TITLE OF THE INVENTION

ANTI-TSG101 ANTIBODIES AND THEIR USES FOR TREATMENT OF VIRAL INFECTIONS

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. Provisional Patent Application No. 60/858,922, filed on November 15, 2006, which is hereby incorporated by reference in its entirety. This application is related to U.S. Patent Application Serial Number

10 11/939,122 filed November 13, 2007, also incorporated herein-by-reference. USSN 11/939,122 is directed to the preparation and use, *inter alia*, of antibodies directed to a family of proteins that are implicated in viral budding, referred to therein as escort proteins. TSG101 is one member of that family. It has a significance all its own.

BACKGROUND OF THE INVENTION

15 ### Field of the Invention

The present invention relates to antibodies that bind a TSG101 protein and inhibit or reduce viral production. The invention also relates to methods of using the TSG101 antibodies for the treatment of viral infections, including HIV infection. The invention further relates to methods of detecting viral infected cells using TSG101 antibodies.

20 ### Background of the Technology

Pathogen and host cell interactions play critical roles in the pathogenesis of viral diseases such as AIDS. For a typical viral infection, viruses have to attach to the host cells through cell surface receptors, fuse with host cell membrane, translocate across the cell membrane, uncoat

viral particles, synthesize and assemble viral proteins using host protein synthesis machinery, and release from host cells through host exporting machinery. The interplay between the viruses and host cells determine the outcome of viral pathogenesis, ranging from the elimination of viruses to a parasitic or lethal infection. For example, HIV employs a variety of strategies to

5   productively infect human cells. A retrovirus, its life cycle begins by attaching to host cells- the primary target is the CD4+ T helper cells and gaining entry via specific receptors. In the cell, the RNA genome is "reverse" transcribed to its complementary DNA, and then shuttled to the nucleus for its integration in the host genome. This integrated "provirus" then directs the production of new viral RNA and proteins, which self-assemble and then "bud" from the cell as

10   mature- and infectious- viral particles, enveloped in plasma membrane. Like all viruses, the HIV is a parasite, unable to catalyze the membrane fission event that drives the budding process. Instead, the nascent virus recruits the cell's membrane sorting machinery to complete this final stage of infection. Such an host and virus interplay has been well demonstrated in individuals, who carry a defective cell surface receptor (CCR5), are completely resistant to HIV infection,

15   elucidating the important roles of host genes and genetic pathways in viral pathogenesis.

Tumor Susceptibility Gene 101 (TSG101, Li, et al., 1996, Cell 85, 319-29) plays important roles in cell growth (Zhong, et al., 1998, Cancer Res. 58, 2699-702; Oh, et al., 2002, Proc. Natl. Acad. Sci. USA 99, 5430-5; Krempler, et al., 2002, J. Biol. Chem. 277, 43216-23; Wagner, et al., 2003, Mol. Cell Biol. 23, 150-62; Li, et al., 1996, Cell 85, 319-29), cellular

20   protein trafficking (Babst, et al., 2000, Traffic 1, 248-58; Bishop, et al., 2002, J. Cell Biol. 157, 91-101), and degradation of p53 (Li, et al., 2001, Proc. Natl. Acad. Sci. USA 98, 1619-24; Ruland, et al., 2001, Proc. Natl. Acad. Sci. USA 98, 1859-64; Moyret-Lalle, et al., 2001, Cancer Res. 61, 486-8). TSG101 is also widely recognized as a key player in this final stage, inhibition of cellular TSG101 blocks the budding process of HIV. Acting in concert with other cellular

25   factors, TSG 101 thus plays an essential role in the budding or spread of HIV viruses. The HIV Gag protein, previously shown to orchestrate viral assembly and budding, binds with high affinity to TSG 101, and this Gag/TSG101 interaction is essential for efficient HIV viral

assembling and budding, as disruption of the Gag/TSG101 interaction prevents HIV viral budding, and significantly limit the spread of HIV virus.

The final step in the assembly of an enveloped virus assembly requires separation of budding particles from the cellular membranes. Three distinct functional domains in Gag,20, i.e., PTAP in HIV-1 (Gottlinger, et al., 1991, Proc. Natl. Acad. Sci. USA 88, 3195-9; Huang, et al., 1995, J. Virol. 69, 6810-8); PPPY in RSV (Parent, et al., 1995, J. Virol. 69, 5455-60), MuLV (Yuan, et al., 1999, Embo. J. 18, 4700-10), and M-PMV (Yasuda, et al., 1998, J. Virol. 72, 4095-103); and YXXL in EIAV (Puffer, et al., 1997, J. Virol. 71, 6541-6), have been identified in different retroviruses that are required for this function and have been termed late, or L domains (Wills, et al., 1991, Aids 5, 639-54). In HIV-1, the L domain contains a PTAP motif and is required for efficient HIV-1 release (see, e.g., Wills, et al., 1994, J. Virol. 68, 6605-6618; Gottlinger, et al., 1991, Proc. Natl. Acad Sci. USA 88, 3195-3199; Huang, et al., 1995, J. Virol. 69, 6810-6818). The L domain of HIV-1 p6, especially the PTAP motif, binds to the cellular protein TSG101 and recruits it to the site of virus assembly to promote virus budding (VerPlank, et al., 2001, Proc. Natl. Acad. Sci. USA, 98:7724-7729; Garrus, et al., 2001, *Cell* 107:55-65; Martin-Serrano, et al., 2001, Nature Medicine 7:1313-19; Pornillos, et al., 2002, EMBO J. 21:2397-2406; Demirov, et al., 2002, Proc. Natl. Acad. Sci. USA 99:955-960; PCT Publication WO 02/072790; U.S. Patent Application Publication No. US 2002/0177207). The UEV domain of TSG101 binds the PTAP motif and mono-ubiquitin (Pornillos, et al., 2002, Embo J. 21, 2397-406; Pornillos, et al., 2002, Nat. Struct. Biol. 9, 812-7), which has also been implicated in HIV-1 budding (Patnaik, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13069-74; Schubert, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13057-62; Strack, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13063-8). Depletion of cellular TSG101 (Garrus, et al., 2001, Cell 107:55-65) or over-expression of a truncated form of TSG101 inhibits HIV-1 release (Demirov, et al., 2002, Proc. Natl. Acad. Sci. USA 99:955-960). Under certain circumstances, TSG101 can even substitute for the HIV-1 L domain to promote virus release (Martin-Serrano, et. al., 2001, Nature Medicine 7:1313-19).

In yeast, the Tsg101 ortholog Vps23 has been shown to interact with Vps28 and Vps37 and to form a protein complex named ESCRT-I, which is critical for endosomal protein sorting into the multivesicular body pathway (Katzmann, et al., 2001, Cell 106, 145-55). It is hypothesized that this intracellular multivesicular body formation resembles HIV-1 release at the

5   plasma membrane (Garrus, et al., 2001, Cell 107:55-65; Patnaik, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13069-74). In mammalian cells, TSG101 interacts with Vps28 to form an ESCRT-I-like complex (Babst, et al., 2000, Traffic 1, 248-58; Bishop, et al., 2002, J. Cell Biol. 157, 91-101; Bishop, et al., 2001, J. Biol. Chem. 276, 11735-42), although the mammalian homolog of Vps37 has not been identified.

10   Recent studies (Blower, et al., 2003, AIDS Rev. 5:113-25; Valdiserri, et al., 2003, Nat. Med. 9:881-6) have estimated that as many as 42 million people worldwide have been infected with HIV. The disease has killed more than 3 million people. While the advent of highly potent and targeted combination therapies has slowed the progression of AIDS in industrialized nations, the AIDS pandemic is causing a "human development catastrophe "in developing nations,

15   particularly in Africa, where more than 21 million Africans have been infected. In South Africa alone, the death toll is projected to rise to 10 million by 2015. Related statistics portend a similar crisis in the Asia Pacific region, which, according to United Nations' estimates, has more than 7 million HIV-infected individuals. Repercussions from the AIDS pandemic extend well beyond the clinic, which lack the resources to treat the swelling numbers of recently infected

20   patients (nearly 20% of the adult population in South Africa is infected). Treatment of HIV-infected and gravely ill AIDS patients is stressing the already over-burdened health care systems of Africa and other developing nations. Worse yet, current treatments for HIV □ despite their initial success in reducing viral load □ are beginning to lose their efficacy, as drug-resistant HIV strains are increasingly isolated in newly infected individuals. Further compounding the

25   therapeutic management of HIV disease is the toxicity of current antiretroviral regimens, the magnitude of which complicates the physician's decision to begin and to maintain treatment. Identifying new therapeutic paradigms for the treatment of HIV disease, especially those with

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mechanisms of action that promise to slow the development of resistance, is indeed a global challenge for the pharmaceutical industry.

Many viruses are also highly mutable. Methods and compositions relying on targeting such viruses directly are normally not sufficient in the treatment of infection by such viruses.

5   For example, HIV-1 is such a highly mutable virus that during the course of HIV-1infection, the antibodies generated in an infected individual do not provide permanent protective effect due in part to the rapid emergence of neutralization escape variants (Thali, et al., 1992, J. Acquired Immune Deficiency Syndromes 5:591-599). Current therapies for the treatment of HIV-infected individuals focus primarily on viral enzymes involved in two distinct stages of HIV infection,

10   the replication of the viral genome and the maturation of viral proteins. Since the virus frequently mutates, strains resistant to an antiviral inhibitor develop quickly, despite the drug's initial therapeutic effects. In one recent study, the percentage of individuals newly infected with drug-resistant HIV strains increased six fold over a five year period (Little, et al., 2002, N. Engl. J. Med. 347:385-94). Further, combination therapy, the current standard of care that attacks HIV

15   with inhibitors of both reverse transcriptase and protease, is leading to the development of multi-drug resistant HIV strains. Anti-retroviral drugs directed against new HIV-based targets, while of considerable value, do not address this increasingly critical issue. For example, HIV strains resistant to Fuzeon® (enfuvirtide), the newest addition to the anti-HIV armamentarium, have already been isolated from patients. Thus, despite its antiviral potency and novel

20   mechanism of action, drug-resistance is likely to undermine the therapeutic potential of viral fusion inhibitors like Fuzeon®. There is therefore a need for developing novel therapeutics and preventative measures to combat viral infections such as HIV infection.

Discussion or citation of a reference herein shall not be construed as an admission that such reference is prior art to the present invention.

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SUMMARY OF THE INVENTION

The present invention provides antibodies that binds to the terminal regions of human TSG101 and methods of using the antibodies for treating viral infections. The present invention also provides methods and compositions for treating viral infection using the anti-TSG101 antibodies.

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In one aspect, the present invention provides monoclonal antibodies that binds to the TSG101 C-terminal region consisting of SEQ ID NO:3. In one embodiment, the antibody is a monoclonal antibody comprising a polypeptide comprising an amino acid sequence recited in SEQ ID NO: 19, 22, 25 or 28.

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In another embodiment, the monoclonal antibodies are antibody D1 and 3G1.

In another embodiment, the antibody is a humanized antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:30-41.

In a preferred embodiment, the humanized antibody comprises either the amino acid sequences recited in SEQ ID NOS:31-35 or the amino acid sequences recited in SEQ ID

15   NOS:36-41.

Another aspect of the present invention relates to a method for treating an enveloped virus infection in a subject. The method comprises administering into the subject an effective amount of the anti-TSG101 antibody of the present invention.

In one embodiment, the enveloped virus is a human immunodeficiency virus, Marburg

20   virus, or Ebola virus.

Yet another aspect of the present invention relates to a pharmaceutical composition for treating human immunodeficiency virus, Marburg virus, and Ebola virus infections. The

composition comprises an effective amount of the anti-TSG101 antibody of the present
invention; and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the 390 amino acid sequence of human TSG101 protein (SEQ ID NO:1).
(GenBank Accession No. U82130.1/GI:1772663).

Figure 2 depicts the effects of anti-TSG101 antibodies on MLV virus production. Left
top panel: phoenix helper cells without treatment of antibody (positive control) showed efficient
production of retroviruses, and infection of N2A target cells; left middle panel: Rabbit IgG had
no effect; left bottom panel: a rabbit antibody against N-terminal TSG101 had an effect of less
than 20% inhibition; right top panel: a rabbit antibody against C-terminal TSG101 significantly
inhibited the production of retroviruses, and infection of

N2A target cells (50 ☐ 70% inhibition); right middle panel: a mixture of anti-C terminal and
anti-N terminal antibodies gave similar results as the anti-C terminal antibody alone; right
bottom panel: N2a cells that were not infected by viruses only showed minimal background
staining.

Figures 3A-E show that GFP-TSG101 localizes to cell surface during viral release. Live
confocal images of Phoenix helper cells with active viral release 24 hours after transfection of
GFP-TSG101. Figure 3A shows bright field images of four cells; Figures 3B-E are live confocal
fluorescence images of the same field at different sections; White arrows point to cell surface
localization of GFP-TSG101.

Figure 4 shows cell surface localization of TSG101 during HIV budding. H9ABgl cells
(CD4+ human T lymphocytes, carrying HIV viral integration) were actively producing and
releasing HIV virions with a defective envelope protein (this non-infectious form of HIV viruses
will not infection other cells, thus specifically allowing the study viral release). The parental H9

cells that do not carry HIV were used as a control. Both H9ABgl and H9 cells were fixed with 2% paraformoldehyde for 10 min. at room temperature (this surface fixation does not permeabilize cells). Anti-TSG101 antibody were incubated with both cell lines for 20 min. and detected with a fluorescence labeled secondary antibody. Top panels: fluorescence images; Bottom panels: bright field images.

Figure 5 shows FACS profile of cell surface localization of TSG101 during HIV budding. Both H9ΔBgl and H9 cells were fixed with 2% paraformoldehyde for 10 min. at room temperature (this surface fixation doesn't permeabilize cells). Anti-TSG101 antibodies were incubated with both cell lines for 20 min. and detected with a fluorescent labeled secondary antibody. The immuno-stained cells were analyzed via FACS. Top panel: H9ΔBgl cells, with 85% cells stained positive for surface TSG101; Bottom panel: H9 control cells, with less than 0.1% cells stained positive for surface TSG101.

Figure 6 shows inhibition of HIV-1 production by anti-TSG101 antibodies. Lane 1 and 5, mock transfection; Lane 2 and 6, pNL4-3 and control antibody (rabbit IgG); Lane 3 and 7, pNL4-3 and anti-TSG101 antibody "B"; Lane 4 and 8, pNL4-3 and anti-TSG101 antibody "E".

Figure 7 shows antibody inhibition of HIV release from H9ΔBgl cells. HIV producing H9ΔBgl cells were incubated with anti-TSGI01 antibody "E" at different concentrations, 48 hours later, viral supernatants were collected and assayed by HIV p24 ELISA kit. Averages of three independent experiments (each with triplicates) were shown. Significant antibody inhibition (* P<0.05) of viral release was observed at 80 ug/ml.

Figure 8 shows antibody inhibition of HIV infectivity. HIV producing Jurkat cells (infected with Wild-type HIV-1) were incubated with anti-TSG101 antibody "E" at 40 ug/ml.

Figures 9A-B show release of Ebola GP and VP40 into culture supernatants. Figure 9A shows 293T cells were transfected with the indicated plasmids, supernatants were cleared from floating and particulate material were pelleted through 20% sucrose by ultracentrifugation. The

individual proteins were detected in the cell lysates and in the particulate material from supernatant by immunoblotting (IB). Figure 9B shows supernatants from cells transfected with Ebola VP40 alone or GP+VP40 were immunoprecipitated with anti-GP mAb and analyzed by immunoblotting. Lower panel shows the expression of VP40 in total cell lysates. IgH: immunoglobulin heavy chain from the antibody used for immunoprecipitation.

Figures 10A-B show electron microscopic analysis of virus like particles generated by EBOV GP and VP40. Particles obtained by ultracentrifugation of the supernatants of 293T cells transfected with Ebola GP+VP40 were negatively stained with uranyl-acetate to reveal the ultrastructure. (Figure 10A) or stained with anti-Ebo-GP mAb followed by Immunogold rabbit anti mouse Ab (Figure 10B), and analyzed by electron microscopy.

Figure 11 shows association of VP40 and TSG101 in 293T cells. Cells were transfected with Myc tagged TSG101 full length (FL) or the indicated truncations along with VP40. After 48h cells were lysed and subjected to immunoprecipitatiopn with anti Myc.

Figure 12 shows Far-Western analysis of association between Ebola VP40 and TSG101 UEV domain.

Figure 13 shows SPR analysis of Ebola VP40 interaction with TSG101.

Figures 14A-B show association of TSG101 with Ebola VLPs and inactivated Ebola virus. In Figure 14, 293 cells were transfected with the indicated plasmids, supernatants were immunoprecipitated with anti-GP mAb and analyzed by immunoblotting with the antibodies indicated on the right. Lower three panel shows the expression of the transfected proteins in total cell lysates. In Figure 14B, 5μg inactivated Ebola virus (iEBOV) were subjected to SDS-PAGE and Western blot analysis with rabbit anti-TSG101 antibody. The molecular weight markers and position of TSG101 are indicated.

Figure 15 shows results of inhibition of Ebola virus release in Hela cells by antiTSG101 antibodies.

Figure 16 is a graph showing the anti-HIV activity of monoclonal antibody pool PE-8 in the form of purified IgG from ascites.

5   Figure 17 is a graph showing the anti-HIV activity of representative subclones of monoclonal antibody pool PE-8 in the form of hybridoma supernatants.

Figure 18 is a graph showing the anti-HIV activity of one of the subclones of monoclonal antibody pool PE-8 in the form of purified IgG from ascites.

Figure 19 is a graph showing inhibition of drug-resistant HIV strains by TSG101

10   dominant negative mutant.

Figure 20 is a graph showing inhibition of drug-resistant HIV strain pL-10R by monoclonal antibody pool PE-8.

Figure 21 is a graph showing inhibition of drug-resistant HIV strain p1617-1 by monoclonal antibody pool PE-8.

15   Figures 22A and 22B are graphs showing inhibition of HIV production in peripheral blood mononuclear cells at day 3 and day 7 post infection, respectively.

Figure 23 is an alignment between the variable region of clone D1 heavy chain and the variable region of a mouse IgG heavy chain.

Figure 24 is an alignment between the variable region of clone D1 light chain and the

20   variable region of a mouse IgG light chain.

Figure 25 is an alignment between the variable region of clone 3G1 heavy chain and the variable region of a mouse IgG heavy chain.

Figure 26 is an alignment between the variable region of clone 3G1 light chain and the variable region of a mouse IgG light chain.

Figure 27 is a graph showing that anti-TSG101 mabs protect mice against EBOV challenge.

5   Figure 28 is a partial listing of viruses identified by researches as dependent or involving some aspect of TSG101 by other researchers in the field.

Figure 29 is a schematic illustration of the preparation of human anti-TSG101 antibodies according to the invention.

Figure 30 graphically reflects the binding of phage candidates to infects cells detected by

10   immunofluorescence.

Figure 31 graphically reflects the binding, by the same TSG101 antibody, to different cell types infected with different viruses.

Figure 32 demonstrates the binding, by staining, of TSG101 antibodies to RSV infected cells, and the absence of binding to controls.

15   Figure 33 reflects, in bar graph format, the ability of the TSG101 antibodies of the invention to kill infected cells through ADCC.

Figure 34 reflects, in bar graph format, the ability of the TSG101 antibodies of the invention to kill virally infected cells and thereby directly inhibit viral infection independent of innate host defense mechanisms

20   **DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides methods of using antibodies that bind a TSG101 protein to inhibit or reduce viral production. The present invention also provides methods of using

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TSG101 antibodies for treating viral infections. The present invention also provides methods and compositions for treating viral infection by targeting TSG101 on the surface of infected cells, e.g., delivering therapeutic and/or diagnostic agents, to such infected cells.

The rational design of therapeutics requires an improved understanding of HIV pathogenesis. Recent studies show that a host protein, TSG101, plays a critical role in the pathogenesis of various viruses such as HIV and Ebola viruses. In particular, TSG101 participates in the process by which viral particles escape, or bud, from infected cells, and therefore represents a novel target for anti-viral drug discovery. Underlying the assembly and release of enveloped RNA viruses from infected cells is a tight coordination between viral and host proteins (Perez, et al., 2001, Immunity 15(5): 687-90; Freed, 2002, J. Virol. 76(10): 4679-87; Pornillos, et al., 2002, Trends Cell Biol. 12(12): 569-79). While many of the protein: protein and protein: membrane interactions that govern the final stages of infection have yet to be identified, the cellular TSG101 protein has emerged as a critical player (Garrus, et al., 2001, Cell 107:55-65; Carter 2002; Pornillos, et al., 2002, Trends Cell Biol. 12 (12): 569-79; Pornillos, et al., 2002, Nat. Struct. Biol. 9, 812-7). Genetic, biochemical and microscopic analyses have shown that TSG101 interacts with multiple enveloped RNA viruses including members of the retrovirus, rhabdovirus and filovirus families.

Despite their considerable evolutionary divergence, many enveloped RNA viruses employ similar strategies to complete the final stages of infection (Martin-Serrano, et. al., 2001, Nature Medicine 7:1313-19; Freed, 2002, J. Virol. 76(10): 4679-87). Of particular importance to Human Immunodeficiency Virus Type 1 (HIV-1), Vesicular Stomatitis Virus (VSV), Ebola Virus (EBOV), Marburg Virus (MARV) and others is the Late or L domain, a sequence motif that uniquely appropriates cellular pathways to drive viral particle assembly and budding. Three sequence motifs with L-domain activity have been characterized: PPxY, YxxL and PTAP (where "x" denotes any amino acid). HIV-1 budding requires the

PTAP motif, found at the amino terminus of the p6Gag protein. Rhabdoviruses, as typified by VSV, utilize the PPxY motif within the Matrix (M) protein. The L-domains of multiple viral families recruit TSG101, a cellular protein critical to endosomal membrane sorting (VerPlank, et al., 2001, Proc. Natl. Acad. Sci. USA 98:7724-7729; Pornillos, et al., 2002, Nat. Struct. Biol. 9,

5   812-7). Initially identified by a random knockout screen in mammalian cells, TSG101 is a 43KDa multifunctional protein involved in membrane trafficking, cell cycle control, microtubule assembly and protein degradation (Li, et al., 1996, Cell 85, 319-29; Bishop, et al., 2001, J. Biol. Chem. 276: 11735-42; Katzmann, et al., 2001, Cell 106, 145-55; Li, et al., 2001, Proc. Natl. Acad. Sci. USA 98, 1619-24. The C-terminus of TSG101 possesses a coiled-coil domain and a

10   domain that auto-regulates its cellular levels; whereas the TSG101 amino-terminus ⬜ which interacts with multiple viral L-domains via a binding pocket that structurally and functionally resembles WW and SH3 domains ⬜ bears significant homology to Ubiquitin Conjugating (UBC) E2 enzymes (Pornillos, et al., 2002, Nat. Struct. Biol. 9, 812-7). Although the UBC-like domain of TSG101 strongly binds ubiquitin, a 76 amino acid protein central to regulating protein

15   turnover and sorting, it lacks the catalytic cysteine residue involved in ubiquitination of target proteins (Hicke, 2001, Cell 106, 527-30).

In eukaryotic cells, TSG101 is a component of ESCRTI (endosomal sorting complex required for transport), a -350kDa cytoplasmic complex that also includes Vps28 and Vps37 (Katzmann, et al., 2001, Cell 106, 145-55; Bishop, et al., 2002, J. Cell Biol. 157, 91-101). The

20   interaction among these three proteins, and their respective roles in membrane trafficking are currently under investigation. The TSG101 yeast homologue, Vps23, was identified by its functional complementation of protein sorting defects (Babst, et al., 2000, Traffic 1, 248-58). Fibroblasts with reduced TSG101 levels and yeast Vps23 null 20 mutants both display defects in the endosomal/MVB pathway. For instance, receptors that would normally enter the MVB

25   system for lysosomal degradation are instead recycled to the surface, leading to profound disturbances in cell signaling. Based on their recent experimental analysis, Katzmann, et al. have suggested that TSG101Nps23 binds ubiquitinated proteins at the surface of early

endosomes, and facilitates their entry into MVB vesicles (Katzmann, et al., 2001, Cell 106, 145-55).

In addition to TSG101, cellular proteins with WW-domains have been shown to interact with the L-domain sequence motifs of enveloped RNA viruses (Harty, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13871-6; Kikonyogo, et al., 2001, Proc. Natl. Acad. Sci. USA 98, 11199-204). For example, Far-Western binding assays have demonstrated a specific interaction with the WW-domains of the mammalian ubiquitin ligase, Nedd4, and its yeast homolog Rsp5, with the VP40 L domain of EBOV (Harty, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13871-6; Kikonyogo, et al., 2001, Proc. Natl. Acad. Sci. USA 98, 11199-204). Indeed, the data thus far point to an important role for ubiquitin in viral budding (Patnaik, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13069-74; Carter, 2002, Trends Microbiol. 10, 203-5; Myers, et al., 2002, J. Virol. 76, 11226-35). There may also be a constitutive interaction between Nedd4 and TSG101. It has been suggested that HIV-1 may exploit Nedd4 and TSG101 to escape from infected cells in a manner wholly unrelated to the endosomal/MVB pathway. Nevertheless, TSG101 is widely regarded as a key host factor appropriated by viruses to drive viral release. The proposed TSG101/ MVB link is based, in part, on the biophysical process of MVB formation, which is known to include the invagination of the endosomal lipid bilayer away from the cytoplasm and towards the lumen (Patnaik, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13069-74; Jasenosky, et al., 2001, J. Virol. 75, 5205-14). Enveloped RNA viruses face similar topological parameters: following viral assembly on the inner leaflet of the membrane, the bilayer mustevaginate towards the extracellular milieu again away from the cytoplasm. Devoid of any catalytic ability to split an otherwise thermodynamically stable bilayer, viruses apparently recruit endosomal membrane factors for assistance. The TSG101: L domain interaction may thus provide a vital nexus between nascent virions and the endosomal machinery that drives membrane fission and budding. As discussed above, TSG101, a constituent of ESCRT-1, sorts ubiquitinated proteins for inclusion in the MVB pathway. But this sorting may be subverted in cells infected with HIV and related enveloped RNA viruses. That is, rather than directing ubiquitinated proteins into the

MVB pathway, TSG101 and its endosomal counterparts may direct the plasma membrane and its associated viral particles to evaginate, forming enveloped vesicles that pinch off from the plasma membrane.

5   The molecular determinants that drive virion assembly and release are still an area of active research, though some general conclusions have emerged. First, the recruitment of TSG101 to the plasma membrane during virion maturation is absolutely required. The data supporting a central TSG101 role are compelling: (i) overexpression of the TSG101 UBC domain trans-dominantly disrupts VLP formation in HIV-1 Gag expressing cells (Demirov, et al., 2002, Proc. Natl. Acad. Sci. USA 99, 955-960); (ii) ablating TSG101 expression via RNA

10   interference impairs HIV-1 budding (Garrus, et al., 2001, Cell 107, 55-65) and (iii) in both of these instances, electron microscopic analysis demonstrated viral particles tethered to the plasma membrane via membranous stalks, structurally similar to those found in cells expressing L-domain defective viruses. As shown by Martin-Serrano, et al., L-domain point mutations that preclude TSG101 binding with the filoviral VP40 or HIV-1 p6Gag, markedly reduce viral

15   particle release from human cells, an effect that coincides with the failure of TSG101 to colocalize with the viral proteins at the lipid bilayer (Martin-Serrano, et. al., 2001, Nature Medicine 7, 1313-19). Related experiments demonstrated that the EBOV L-domain was able to substitute for the p6Gag L-domain, with no discernible effects on VLP release, underscoring the conserved nature of the enveloped RNA viral budding mechanisms. Significantly, HIV-1

20   L-domain is dispensable once TSG101 is directed fused to HIV-1 Gag. Therefore the primary responsibility of the L-domain is to recruit TSG101 to the plasma membrane. This interaction between TSG101 and viral L domains represents a novel target for the prevention and treatment of HIV, EBOV and MARV infections (Luban, 2001, Nat. Med. 7, 1278-80; Senior, 2001, Drug Discov. Today 6, 1184-1186).

25   The inventor has discovered that anti-TSG101 antibodies can be used for inhibiting or reducing viral infections.

ANTI-TSG101 ANTIBODIES

The invention encompasses the use of an antibody that contains a binding site which specifically binds a TSG101 protein for inhibiting or reducing viral infection. Such anti-TSG101 antibodies can therefore be used as broad spectrum anti-viral agents. The term "antibody" as used herein refers to immunoglobulin molecules. In one embodiment, the antibody binds a C-terminal region of a TSG101 protein. In a preferred embodiment, the antibody binds an epitope comprised in the amino acid region QLRALMQKARKTAGLSDLY (SEQ ID NO:3). In another preferred embodiment, the antibody is a monoclonal antibody comprising a polypeptide comprising an amino acid sequence recited in SEQ ID NO: 19, 22, 25 or 28. In a more preferred embodiment, the monoclonal antibodies are antibody D1 and 3G1. In another preferred embodiment, the antibody is a humanized antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:30-41. In a more preferred embodiment, the humanized antibody comprises either the amino acid sequences recited in SEQ ID NOS:31-35 or the amino acid sequences recited in SEQ ID NOS:36-41.

In another embodiment, the antibody binds an N-terminal region of a TSG101 protein. In a preferred embodiment, the antibody binds an epitope comprised in the amino acid region VRETVNVITLYKDLKPVL (SEQ ID NO:2).

As used herein, "epitope" refers to an antigenic determinant, i.e., a region of a molecule that provokes an immunological response in a host or is bound by an antibody. This region can but need not comprise consecutive amino acids. The term epitope is also known in the art as "antigenic determinant." An epitope may comprise as few as three amino acids in a spatial conformation which is unique to the immune system of the host. Generally, an epitope consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods for determining the spatial conformation of such amino acids are known in the art.

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The invention also envisions the use of antibody fragments that contain a binding site which specifically binds a TSG101 protein. Examples of immunologically active fragments of immunoglobulin molecules include F(ab) and F(ab')2 fragments which can be generated by treating the antibody with an enzyme such as pepsin or papain. Examples of methods of generating and expressing immunologically active fragments of antibodies can be found in U.S. Patent No. 5,648,237 which is incorporated herein by reference in its entirety.

The immunoglobulin molecules are encoded by genes which include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant regions, as well as a myriad of immunoglobulin variable regions. Light chains are classified as either kappa or lambda. Light chains comprise a variable light (V_L) and a constant light (C_L) domain. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes IgG, IgM, IgA, IgD and IgE, respectively. Heavy chains comprise variable heavy (V_H), constant heavy 1 (CH1), hinge, constant heavy 2 (CH2), and constant heavy 3 (CH3) domains. The IgG heavy chains are further sub-classified based on their sequence variation, and the subclasses are designated IgGI, IgG2, IgG3 and IgG4.

Antibodies can be further broken down into two pairs of a light and heavy domain. The paired V_L and V_H domains each comprise a series of seven subdomains: framework region 1 (FRI), complementarity determining region 1 (CDR1), framework region 2 (FR2), complementarity determining region 2 (CDR2), framework region 3 (FR3), complementarity determining region 3 (CDR3), framework region 4 (FR4) which constitute the antibody-antigen recognition domain.

A chimeric antibody may be made by splicing the genes from a monoclonal antibody of appropriate antigen specificity together with genes from a second human antibody of appropriate biologic activity. More particularly, the chimeric antibody may be made by splicing the genes encoding the variable regions of an antibody together with the constant region genes from a second antibody molecule. This method is used in generating a humanized monoclonal antibody

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wherein the complementarity determining regions are mouse, and the framework regions are human thereby decreasing the likelihood of an immune response in human patients treated with the antibody (U.S. Patent Nos. 4,816,567, 4,816,397, 5,693,762; 5,585,089; 5,565,332 and 5,821,337 which are incorporated herein by reference in their entirety).

5   An antibody suitable for use in the present invention may be obtained from natural sources or produced by hybridoma, recombinant or chemical synthetic methods, including modification of constant region functions by genetic engineering techniques (U.S. Patent No. 5,624,821). The antibody of the present invention may be of any isotype, but is preferably human IgG1.

10   Antibodies exist for example, as intact immunoglobulins or can be cleaved into a number of well-characterized fragments produced by digestion with various peptidases, such as papain or pepsin. Pepsin digests an antibody below the disulfide linkages in the hinge region to produce a F(ab)'2 fragment of the antibody which is a dimer of the Fab composed of a light chain joined to a VH-CHI by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the 15   disulfide linkage in the hinge region thereby converting the F(ab)'2 dimer to a Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region. See Paul, ed., 1993, Fundamental Immunology, Third Edition (New York: Raven Press), for a detailed description of epitopes, antibodies and antibody fragments. One of skill in the art will recognize that such Fab' fragments may be synthesized *de novo* either chemically or using recombinant DNA technology. 20   Thus, as used herein, the term antibody fragments includes antibody fragments produced by the modification of whole antibodies or those synthesized *de novo*.

As used herein, an antibody can also be a single-chain antibody (scFv), which generally comprises a fusion polypeptide consisting of a variable domain of a light chain fused via a polypeptide linker to the variable domain of a heavy chain.

The invention also encompasses the use of a polyclonal population of anti-TSG101 antibodies for inhibiting or reducing viral infection. As used herein, a polyclonal population of anti-TSGI01 antibodies of the present invention refers to a population of anti-TSG101 antibodies, which comprises a plurality of different anti-TSG101 antibodies each having a

5   different binding specificity. In one embodiment, the population of anti-TSG101 antibodies comprises antibodies that bind a C-terminal region of a TSG101 protein. In a preferred embodiment, the population of anti-TSG101 antibodies comprises antibodies that bind one or more epitopes comprised in the amino acid region QLRALMQKARKTAGLSDLY (SEQ ID NO:3). In another embodiment, the population of anti-TSG101 antibodies comprises antibodies

10   that bind an N-terminal region of a TSG101 protein. In a specific embodiment, the population of anti-TSG101 antibodies comprises antibodies that bind one or more epitopes comprised in the amino acid region VRETVNVITLYKDLKPVL (SEQ ID NO:2).

Preferably, the plurality of anti-TSG101 antibodies of the polyclonal population includes specificities for different epitopes of TSG101 protein. In preferred embodiments, at least 90%,

15   75%, 50%, 20%, 10%, 5%, or 1% of anti-TSG101 antibodies in the polyclonal population target the desired epitopes. In other preferred embodiments, the proportion of any single anti-TSG101 antibody in the polyclonal population does not exceed 90%, 50%, or 10% of the population. The polyclonal population comprises at least 2 different anti-TSG101 antibodies with different specificities. More preferably, the polyclonal population comprises at least 10 different

20   anti-TSG101 antibodies. Most preferably, the polyclonal population comprises at least 100 different anti-TSG101 antibodies with different specificities.

PRODUCTION OF ANTI-TSG101 ANTIBODIES

TSG101 protein or a fragment thereof can be used to raise antibodies which bind TSG101 protein. Such antibodies include but are not limited to polyclonal, monoclonal,

25   chimeric, single chain, Fab fragments, and an Fab expression library. In a preferred embodiment,

anti C-terminal TSG101 antibodies are raised using an appropriate C-terminal fragment of a TSG101 protein. Such antibodies are useful in inhibiting viral production.

PRODUCTION OF MONOCLONAL ANTI-TSG101 ANTIBODIES

        Antibodies can be prepared by immunizing a suitable subject with a TSG101 protein or a
5   fragment thereof as an immunogen. The antibody titer in the immunized subject can be
monitored over time by standard techniques, such as with an enzyme linked immunosorbent
assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules can be
isolated from the mammal (e.g., from the blood) and further purified by well-known techniques,
such as protein A chromatography to obtain the IgG fraction. In one embodiment, a polyclonal
10   anti-N terminal TSG101 antibody (also referred to as anti-TSG101 antibody "C") is raised using
an N-terminal fragment of the human TSG101 protein: VRETVNVITLYKDLKPVL (SEQ ID
NO:2). In another embodiment, a polyclonal anti-C terminal TSG101 antibody (also referred to
as anti-TSG101 antibody "E") is raised using a C-terminal fragment of the human TSG101
protein: QLRALMQKARKTAGLSDLY (SEQ ID NO:3). In yet another embodiment,
15   monoclonal anti-C terminal TSG101 antibody (e.g., pool PE-8, mab D1, and mab 3G1) are
raised using a C-terminal fragment of the human TSG101 protein (SEQ ID NO:3).

        At an appropriate time after immunization, e.g., when the specific antibody titers are
highest, antibody-producing cells can be obtained from the subject and used to prepare
monoclonal antibodies by standard techniques, such as the hybridoma technique originally
20   described by Kohler and Milstein (1975, Nature 256:495-497), the human B cell hybridoma
technique by Kozbor, et al. (1983, Immunol. Today 4:72), the EBV-hybridoma technique by
Cole, et al. (1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or
trioma techniques. The technology for producing hybridomas is well known (see Current
Protocols in Immunology, 1994, John Wiley & Sons, Inc., New York, NY). Hybridoma cells
25   producing a monoclonal antibody of the invention are detected by screening the hybridoma

culture supernatants for antibodies that bind the polypeptide of interest, e.g., using a standard ELISA assay.

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler, et al., 1975, Nature, 256:495, or may be made by recombinant DNA methods (U.S. Patent. No. 4,816,567). The term "monoclonal antibody" as used herein also indicates that the antibody is an immunoglobulin.

In the hybridoma method of generating monoclonal antibodies, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization (see, e.g., U.S. Patent No. 5,914,112, which is incorporated herein by reference in its entirety).

Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986)). The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-ll mouse tumors available from the Salk

5   Institute Cell Distribution Center, San Diego, Calif USA, and SP-2 cells available from the American Type Culture Collection, Rockville, Md. USA.

Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, 1984, J. Immunol., 133:3001; Brodeur, et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63

10   (Marcel Dekker, Inc., New York, 1987)). Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immuno-absorbent assay (ELISA). The binding affinity of the monoclonal

15   antibody can, for example, be determined by the Scatchard analysis of Munson, et al., 1980, Anal. Biochem., 107, 220.

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103,

20   Academic Press, 1986). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal. The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel

25   electrophoresis, dialysis, or affinity chromatography.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody directed against a TSG101 protein or a fragment thereof can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the TSG101 protein or the fragment. Kits for generating and screening

5   phage display libraries are commercially available (e.g., Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene antigen SurfZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent Nos. 5,223,409 and 5,514,548; PCT Publication No. WO 92/18619; PCT

10   Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs, et al., 1991, Bio/Technology 9:1370-1372; Hay, et al., 1992, Hum. Antibod. Hybridomas 3, 81-85; Huse, et al., 1989, Science 246, 1275-1281; Griffiths, et al., 1993, EMBO J. 12, 725-734.

15   Functional Genetics, Inc., assignee of this patent application, of Gaithersburg, Maryland derived a panel of antibodies that selectively target TSG101 on virally-infected cells. In brief summary, a naïve scFv library was isolated from an anonymous human donor and was used to generate a standard phage library. TSG101 immunoreactivity was determined by immobilizing purified, full-length TSG101, or polypeptides encompassing different regions of TSG101 and then

20   isolating phage that bound immobilized TSG101 using an ELISA-based format in situ.

As shown in Figure 29, after multiple rounds of screening, the resulting phage-based scFv candidates were screened for their abilities to bind TSG101 that is uniquely exposed on the surface of virally-infected cells. These studies utilized flow cytometric assessment of labeled cells to provide an objective analysis of phage binding to infected cells. Two different virus types (HIV and

25   influenza) were used to assess phage that bind TSG101 on the surface of infected cells. Non-infected cells provided a negative control. These assays identified scFv, encoded in phage, which

demonstrated the ability to bind virus-infected cells. Figure 30.

Those phage encoded scFv candidates that selectively recognized the surface of virus-infected cells were modified to engineer the scFv into full length IgG1 antibodies. The resulting antibodies were then screened using the same criteria as indicated above. The antibodies derived from the CB8 phage provide a specific example.

As a further indication of specificity, all of the resulting antibodies or phage candidates were evaluated for their binding to different regions of TSG101 (using the ELISA techniques detailed above). Most of the candidates, including CB8, recognized the C-terminal region of TSG101 while at least one candidate recognized an epitope in the UEV region.

CB8 antibodies were able to recognize the surface of virus infected cells. For example, CB8 antibodies selectively recognized human Hep2 cells that had been infected with influenza virus. Likewise, these antibodies were broad-spectrum in their recognition of cells infected by different viruses as the same antibodies recognized human MT4 T lymphocyte cells that had been infected with HIV. Figure 31. Similarly, these antibodies selectively recognized non-human cell models that had been infected with virus, including canine MDCK cells infected with influenza and primate Vero cells that had been infected with Ebola virus.

Functional Genetics, Inc. maintains pure stocks of antibodies C, D1, E, 3G1 and CB8 as described above, coded to those designations. Deposits of these antibodies are being prepared, as of November 15, 2007 for maintenance at the ATCC under Budapest Treaty Conditions as Deposits_____, _____, _____,_____ and _____. Until such time as the availability of these deposits can be confirmed, these antibodies are available from Functional Genetics, Inc. under the terms set forth in the Budapest Treaty for biologic materials including antibodies that are the subject of a pending patent application.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison, et al., 1984, Proc. Natl. Acad. Sci., 81, 6851-6855; Neuberger, et al., 1984, Nature 312, 604-608;

Takeda, et al., 1985, Nature, 314, 452-454) by splicing the genes from a mouse antibody

molecule of appropriate antigen specificity together with genes from a human antibody molecule

of appropriate biological activity can be used. A chimeric antibody is a molecule in which

different portions are derived from different animal species, such as those having a variable

5   region derived from a murine mAb and a human immunoglobulin constant region. (See, e.g.,

Cabilly, et al., U.S. Patent No. 4,816,567; and Boss, et al., U.S. Patent No. 4,816,397, which are

incorporated herein by reference in their entirety.)

       Humanized antibodies are antibody molecules from non-human species having one or

more complementarity determining regions (CDRs) from the non-human species and a

10   framework region from a human immunoglobulin molecule. (see e.g., U.S. Patent No. 5,585,089,

which is incorporated herein by reference in its entirety.) Such chimeric and humanized

monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for

example using methods described in PCT Publication No. WO 87/02671; European Patent

Application 184,187; European Patent Application 171,496; European Patent Application

15   173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567 and 5,225,539;

European Patent Application 125,023; Better, et al., 1988, Science 240:1041-1043; Liu, et al.,

1987, Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu, et al., 1987, J. Immunol. 139:3521-3526;

Sun, et al., 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura, et al., 1987, Canc. Res.

47:999-1005; Wood, et al., 1985, Nature 314, 446-449; Shaw, et al., 1988, J. Natl. Cancer Inst.

20   80, 1553-1559; Morrison 1985, Science 229:1202-1207; Oi, et al., 1986, Bio/Techniques 4, 214;

Jones, et al., 1986, Nature 321, 552-525; Verhoeyan, et al., 1988, Science 239, 1534; and

Beidler, et al., 1988, J. Immunol. 141, 4053-4060.

       Complementarity determining region (CDR) grafting is another method of humanizing

antibodies. It involves reshaping murine antibodies in order to transfer full antigen specificity

25   and binding affinity to a human framework (Winter, et al. U.S. Patent No. 5,225,539).

CDR-grafted antibodies have been successfully constructed against various antigens, for

example, antibodies against IL-2 receptor as described in Queen, et al., 1989 (Proc. Natl. Acad. Sci. USA 86, 10029); antibodies against cell surface receptors-CAMPATH as described in Riechmann, et al. (1988, Nature, 332, 323; antibodies against hepatitis B in Cole, et al. (1991, Proc. Natl. Acad. Sci. USA 88, 2869); as well as against viral antigens-respiratory syncitial virus in Tempest, et al. (1991, Bio-Technology 9, 267). CDR-grafted antibodies are generated in which the CDRs of the murine monoclonal antibody are grafted into a human antibody. Following grafting, most antibodies benefit from additional amino acid changes in the framework region to maintain affinity, presumably because framework residues are necessary to maintain CDR conformation, and some framework residues have been demonstrated to be part of the antigen binding site. However, in order to preserve the framework region so as not to introduce any antigenic site, the sequence is compared with established germline sequences followed by computer modeling.

Completely human antibodies, such as CB8, are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a TSG101 protein.

Monoclonal antibodies directed against a TSG101 protein can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13, 65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see e.g., U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc.

(Freemont, CA, see, for example, U.S. Patent No. 5,985,615) and Medarex, Inc. (Princeton, NJ), can be engaged to provide human antibodies directed against a TSG101 protein or a fragment thereof using technology similar to that described above.

Completely human antibodies which recognize and bind a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers, et al., 1994, Biotechnology 12, 899-903).

A pre-existing anti-TSG101 antibody can be used to isolate additional antigens of the pathogen by standard techniques, such as affinity chromatography or immunoprecipitation for use as immunogens. Moreover, such an antibody can be used to detect the protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of TSG101 protein. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include 125I, 131I, 35S or 3H.

PRODUCTION OF POLYCLONAL ANTI-TSG101 ANTIBODIES

The anti-TSG101 antibodies can be produced by immunization of a suitable animal, such as but are not limited to mouse, rabbit, and horse.

An immunogenic preparation comprising a TSG101 protein or a fragment thereof are

5   used to prepare antibodies by immunizing a suitable subject (e.g., rabbit, goat, mouse or other mammal). An appropriate immunogenic preparation can contain, for example, recombinantly expressed or chemically synthesized TSG101 peptide or polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent.

10   A fragment of a TSG101 protein suitable for use as an immunogen comprises at least a portion of the TSG101 protein that is 8 amino acids, more preferably 10 amino acids and more preferably still, 15 amino acids long.

The invention also provides chimeric or fusion TSG101 polypeptides for use as immunogens. As used herein, a "chimeric" or "fusion" TSG101 polypeptides comprises all or

15   part of a TSG101 polypeptide operably linked to a heterologous polypeptide. Within the fusion TSG101 polypeptide, the term "operably linked" is intended to indicate that the TSG101 polypeptide and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of the TSG101 polypeptide.

20   One useful fusion TSG101 polypeptide is a GST fusion TSG101 polypeptide in which the TSG101 polypeptide is fused to the C-terminus of GST sequences. Such fusion TSG101 polypeptides can facilitate the purification of a recombinant TSG101 polypeptide.

In another embodiment, the fusion TSG101 polypeptide contains a heterologous signal sequence at its N-terminus so that the TSG101 polypeptide can be secreted and purified to high

homogeneity in order to produce high affinity antibodies. For example, the native signal sequence of an immunogen can be removed and replaced with a signal sequence from another protein. For example, the gp67 secretory sequence of the baculovirus envelope protein can be used as a heterologous signal sequence (Current Protocols in Molecular Biology, Ausubel, et al.,

5   eds., John Wiley & Sons, 1992). Other examples of eukaryotic heterologous signal sequences include the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene; La Jolla, California). In yet another example, useful prokaryotic heterologous signal sequences include the phoA secretory signal and the protein A secretory signal (Pharmacia Biotech; Piscataway, New Jersey).

10   In yet another embodiment, the fusion TSG101 polypeptide is an immunoglobulin fusion protein in which all or part of a TSG101 polypetide is fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin fusion proteins can be used as immunogens to produce antibodies directed against the TSG101polypetide in a subject.

           Chimeric and fusion TSG101 polypeptide can be produced by standard recombinant

15   DNA techniques. In one embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (e.g., Ausubel, et al., supra). Moreover, many expression

20   vectors are commercially available that already encode a fusion domain (e.g., a GST polypeptide). A nucleic acid encoding an immunogen can be cloned into such an expression vector such that the fusion domain is linked in-frame to the polypeptide.

           The TSG101 immunogenic preparation is then used to immunize a suitable animal. Preferably, the animal is a specialized transgenic animal that can secret human antibody.

25   Non-limiting examples include transgenic mouse strains which can be used to produce a polyclonal population of antibodies directed to a specific pathogen (Fishwild, et al., 1996, Nature

Biotechnology 14, 845-851; Mendez, et al., 1997, Nature Genetics 15, 146-156). In one embodiment of the invention, transgenic mice that harbor the unrearranged human immunoglobulin genes are immunized with the target immunogens. After a vigorous immune response against the immunogenic preparation has been elicited in the mice, the blood of the

5   mice are collected and a purified preparation of human IgG molecules can be produced from the plasma or serum. Any method known in the art can be used to obtain the purified preparation of human IgG molecules, including but is not limited to affinity column chromatography using anti-human IgG antibodies bound to a suitable column matrix. Anti-human IgG antibodies can be obtained from any sources known in the art, e.g., from commercial sources such as Dako

10   Corporation and ICN. The preparation of IgG molecules produced comprises a polyclonal population of IgG molecules that bind to the immunogen or immunogens at different degree of affinity. Preferably, a substantial fraction of the preparation are IgG molecules specific to the immunogen or immunogens. Although polyclonal preparations of IgG molecules are described, it is understood that polyclonal preparations comprising any one type or any combination of

15   different types of immunoglobulin molecules are also envisioned and are intended to be within the scope of the present invention.

A population of antibodies directed to a TSG101 protein can be produced from a phage display library. Polyclonal antibodies can be obtained by affinity screening of a phage display library having a sufficiently large and diverse population of specificities with a TSG101 protein

20   or a fragment thereof. Examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent Nos. 5,223,409 and 5,514,548; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO

25   92/09690; PCT Publication No. WO 90/02809; Fuchs, et al., 1991, Biotechnology 9, 1370-1372; Hay, et al., 1992, Hum. Antibod. Hybridomas 3, 81-85; Huse, et al., 1989, Science 246, 1275-1281; Griffiths, et al., 1993, EMBO J. 12, 725-734. A phage display library permits

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selection of desired antibody or antibodies from a very large population of specificities. An additional advantage of a phage display library is that the nucleic acids encoding the selected antibodies can be obtained conveniently, thereby facilitating subsequent construction of expression vectors.

5   In other preferred embodiments, the population of antibodies directed to a TSG101 protein or a fragment thereof is produced by a method using the whole collection of selected displayed antibodies without clonal isolation of individual members as described in U.S. Patent No. 6,057,098, which is incorporated by reference herein in its entirety. Polyclonal antibodies are obtained by affinity screening of a phage display library having a sufficiently large repertoire

10   of specificities with, e.g., an antigenic molecule having multiple epitopes, preferably after enrichment of displayed library members that display multiple antibodies. The nucleic acids encoding the selected display antibodies are excised and amplified using suitable PCR primers. The nucleic acids can be purified by gel electrophoresis such that the full length nucleic acids are isolated. Each of the nucleic acids is then inserted into a suitable expression vector such that a

15   population of expression vectors having different inserts is obtained. The population of expression vectors is then expressed in a suitable host.

IDENTIFYING ANTI-TSG101 ANTIBODIES THAT INHIBIT VIRAL PRODUCTION

The invention provides a method for identifying anti-TSG101 antibodies that can be used to inhibit or reduce viral budding. In one embodiment, the invention provides a method for

20   determining the effect of anti-TSG101 antibodies on viral infections using a retroviral infection assay. A murine leukemia virus (MLV) derived vector which contains an E. *coli lacZ* gene expressed from the long terminal repeat (LTR) promoter(pBMN-Z-INeo) is transfected into an amphotropic murine leukemia retroviral packaging cell line derived from 293 cells (Phoenix A, ATCC). Retroviruses produced by the Phoenix A helper cells are collected and used to infect a

25   mouse N2A cells (ATCC). Anti-TSG101 antibodies are added to 293 helper cells 24 hours after the transfection of the MLV vector. The effectiveness of TSG101 antibodies on viral production

is determined by the efficiency of viral supernatant to infect the target cells (N2A). The

infection of N2A cells is then determined by cellular staining of (3-galactosidase activity

(positive cells are stained blue, shown as dark spots in Figure 2).

Typically, phoenix A cells are seeded on poly-D-lysine coated 6-well plate a day before

transfection. Four microgram of pBMN-Z-I-Neo is then transfected into each well in the

presence of 12 ul of Lipofectamine 2000 (Invitrogen). Twenty-four hours post-transfection,

media are replaced with 1 ml/well of fresh media containing trichostatin A (3 uM) and 5 or 10 ug

of proper anti-TSG 101 antibodies. 24 to 48 hours later, viral supernatants are collected, filtered

with 0.2 um filters, and 1 ml of viral supernatant is mixed with 1 ml of fresh media containing

polybrene (10 ug/ml), and then is used to infect one well of N2a cells. 48 hours post-infection,

N2a cells are fixed and stained with X-Gal as described in the 13-Gal staining kit (Invitrogen).

Results are documented by digital images. Preferably, anti-TSG101 antibodies that reduce viral

production by at least 10%, 20%, 50%, 70% or 90% are identified.

In the following exemplary experiments, the two anti-TSG101 antibodies "C" and "E" are

tested for their effect on viral infection. Rabbit IgG is used as non-specific antibody control.

More than 10 independent experiments are performed, and representative results are shown in

Figure 2. Phoenix helper cells without treatment of antibody (positive control) showed efficient

production of retroviruses, and infection of N2A target cells (left top panel); Rabbit IgG had no

effect (left middle panel). The anti-TSG101 antibody "C" reduced viral production by about

20%-60% (left bottom panel). The anti-TSG101 antibody "E" reduced viral production by about

50 - 70% (right top panel). A mixture of anti-C-terminal and anti-N-terminal antibodies give

similar results as the anti-C terminal antibody alone (Right middle panel). N2a cells that are not

infected by viruses only showed minimal background staining (right bottom panel).

In another embodiment, anti-TSG101 antibodies that can be used to inhibit or reduce

viral budding are identified based on their binding to cell surface TSG101, e.g., in a human

CD4+ human T cell line H9 transfected with HIV (designated as H9ΔBg1). H9ΔBg1 cells are

human CD4+ T lymphocytes transfected with an envelop-defective HIV construct (deletion of a

Bgl II fragment of HIV genome). The stably transfected H9ABgl cells produce a non-infectious

form of HIV due to the defective HIV envelop, hence cannot infect other H9ΔBg1 cells in the

culture. In one embodiment, the untransfected H9 cells are used as control. Anti-TSG101

5   antibodies that bind to H9ΔBg1 but not the untransfected H9 cells are identified as the antibodies

that can be used to inhibit or reduce viral budding.

In a preferred embodiment, binding of an anti-TSG101 antibody to cell surface TSG101

in HIV producing cells (e.g., H9ΔBgl) and control H9 cells is identified by Fluorescence

Activated Cell Sorting (FACS). In one embodiment, both H9ΔBg1 and H9 cells are fixed,

10   incubated with anti-TSG101 antibodies, and then stained with a fluorescence labeled secondary

antibody. The immuno-stained cells are then analyzed by FACS.

In another embodiment, a HIV-1 viral production assay is used to further examine the

inhibitory effect of TSG101 on retroviral production. The HIV-1 vector pNL4-3 is transfected

into 293T cells. 24 hours after transfaction, an anti-TSG101 antibody and, optionally, a

15   non-specific control antibody are added respectively into the cell cultures. After additional 24

hours incubation, cell lysates are extracted, cell culture supernatants are collected and HIV-1

virions are purified, e.g., by sucrose gradients. Both cell lysates and purified virions are

analyzed by Western blot using, e.g., anti-HIV-1 antibodies such as anti-p55 and/or anti-p24

antibodies. Anti-TSG101 antibody that exhibit significant inhibition of HIV-1 virion release

20   (e.g., more than 40%, 50%, 60%, 70%, or 80% inhibition by density tracing of the Western

blots) can be identified.

In still another embodiment, the effect of an anti-TSG101 antibody on HIV release is

evaluated using a HIV release assay based on H9ΔBg1 cells. HIV release from H9ΔBg1 cells

can be directly measured by HIV p24 ELISA of cell culture supernatant. In one embodiment, a

25   plurality of different concentrations of an anti-TSG101 antibody is incubated respectively with

H9ΔBg1 cells. In one embodiment, a control antibody (e.g., rabbit IgG at the same

concentrations) is also incubated respectively with H9ΔBg1 cells. 48 hours after antibody

addition, culture supernatants are collected for HIV p24 ELISA. Effect of the anti-TSG101

antibody for inhibition of viral release is then determined by comparingdata of the anti-TSG101

antibody with the data of the corresponding control antibody.

5   In still another embodiment, the effect of an anti-TSG101 antibody on HIV infectivity

following viral release is determined. In one embodiment, HIV supernatants from Jurkat cells

are used to infect MAGI cells in the presence of an anti-TSG101 antibody. Rabbit IgG can be

used as controls. The anti-TSG101 antibody's effect on HIV infectivity is determined by

comparing with the control.

10   USES OF ANTI-TSG101 ANTIBODIES FOR TREATMENT OF VIRAL INFECTIONS

           TSG101 antibodies are effective in inhibiting viral production. The invention therefore

provides a method of treating viral infections, including HIV infection, using TSG101

antibodies, e.g., anti-C-terminal TSG101 antibodies.

VIRAL INFECTIONS

15   Diseases or disorders that can be treated or prevented by the use of an anti-TSG101

antibody of the present invention include, but are not limited to, those caused by a ritrovirus,

rhabdovirus, or filovirus, hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella,

adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest,

rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus,

20   cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsachie virus, mumps virus, measles

virus, rubella virus, polio virus, human immunodeficiency virus type I (HIV-I), and human

immunodeficiency virus type II (HIV-II), any picornaviridae, enteroviruses, caliciviridae, any of

the Norwalk group of viruses, togaviruses, alphaviruses, flaviviruses, such as Dengue virus,

coronaviruses, rabies virus, Marburg viruses, Ebola viruses, parainfluenza virus,

25   orthomyxoviruses, bunyaviruses, arenaviruses, reoviruses, rotaviruses, orbiviruses, human T cell

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leukemia virus type I, human T cell leukemia virus type II, simian immunodeficiency virus, lentiviruses, polyomaviruses, parvoviruses, Epstein-Barr virus, human herpesvirus-6, cercopithecine herpes virus 1 (B virus), and poxviruses.

Additional diseases or disorders that can be treated or prevented by the use of an anti-TSG101 antibody of the present invention include, but are not limited to, those caused by influenza virus, human respiratory syncytial virus, pseudorabies virus, pseudorabies virus II, swine rotavirus, swine parvovirus, bovine viral diarrhea virus, Newcastle disease virus h, swine flu virus, swine flu virus, foot and mouth disease virus, hog colera virus, swine influenza virus, African swine fever virus, infectious bovine rhinotracheitis virus, infectious laryngotracheitis virus, La Crosse virus, neonatal calf diarrhea virus, Venezuelan equine encephalomyelitis virus, punta toro virus, murine leukemia virus, mouse mammary tumor virus, equine influenza virus or equine herpesvirus, bovine respiratory syncytial virus or bovine parainfluenza virus.

METHODS OF USING ANTI-TSG101 ANTIBODIES FOR INHIBITING VIRAL RELEASE

In one embodiment, the present invention provides methods of using anti-TSG101 antibodies, preferably anti-C-terminal TSG101 antibodies, in inhibiting or reducing viral budding, such as HIV-1 budding, from infected mammalian cells. In the methods of the invention, one or more anti-TSG101 antibodies are allowed to contact an infected cell. The anti-TSG101 antibodies binds to the TSG101 protein on the surface of the infected cell. The binding of the anti-TSG101 antibodies inhibits or reduces the release, or budding, of viral particles from the cell.

In another embodiment, the present invention thus also provides methods using anti-TSG101 antibodies, preferably anti-C-terminal TSG101 antibodies, for treating infection by an enveloped virus, e.g., HIV-1, in a mammal, e.g., a human. In the methods of the invention, one or more anti-TSG101 antibodies can be administered to an mammal, e.g., a human, infected

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by the virus. After administration, the anti-TSG101 antibodies bind to TSG101 protein on the surface of an infected cell and inhibiting viral budding from the infected cell.

In still another embodiment of the invention, the anti-TSG101 antibodies, preferably anti-C-terminal TSG101 antibodies are used in conjunction with one or more other therapeutic

5   anti-viral drugs. In such combined therapies, the anti-TSG101 antibodies can be administered before, at the same time, or after the administration of the therapeutic drugs. The time intervals between the administration of the anti-TSG101 antibodies and the therapeutic drugs can be determined by routine experiments that are familiar to one skilled in the art.

In still another embodiment, the present invention provides a method for treatment of

10   viral infection using an anti-TSG101 antibody, e.g., an anti-C-terminal TSG101 antibody, that belongs to an isotype that is capable of mediating the lysis of infected cells to which the anti-TSG101 antibody is bound. In a preferred embodiment, the anti-TSG101 antibody belongs to an isotype that binds a growth factor receptor and activates serum complement and/or mediates antibody dependent cellular cytotoxicity (ADCC) by activating effector cells, e.g.,

15   macrophages. In another preferred embodiment, the isotype is IgG1, IgG2a, IgG3 or IgM.

In this respect, as a further indication of activity of an antibody-based drug, the antibody candidates have been screened for their ability to selectively kill virus-infected cells. For this, target cells were labeled with a vital dye. These cells were then infected with virus and subjected to ADCC assays, using NK cells derived from normal donors as effector cells. These studies

20   demonstrated robust and selective killing of virally infected cells by CB8 antibodies. Figure 33. These antibodies were quite potent, as evidenced by the fact that at least 70% of infected cells could be killed at antibody concentrations of 125 ng/mL of CB8 antibody and at relatively low effector:target ratios (3:1). This remarkable selective killing of virally-infected cells has been reproduced with multiple and different target cells types (Hep2, MT4, MDCK), multiple and

25   different donors (of NK cells) and multiple and different viruses (HIV, Influenza).

Using other TSG101 antibodies, we have demonstrated that antibody targeting of TSG101 can directly inhibit viral infection independent of innate host defense mechanisms (ADCC or CDC). This finding is consistent with evidence that TSG101 interactions with viral late domain proteins are essential for the propagation of many different viruses (see Figure 34).

5   These results lead us to anticipate that many or all of the antibody candidates listed above could have the ability to directly inhibit viral infection by blocking critical interactions.

The dosage of the anti-TSG101 antibodies can be determined by routine experiments that are familiar to one skilled in the art. The effects or benefits of administration of the anti-TSG101 antibodies can be evaluated by any methods known in the art. The compounds could be used

10   alone or in combination with the current standards of care for any of the viruses indicated above. In general, although other modes of administration are contemplated, IV or IM injection, or sustained IV administration, are preferred routes. Dosages will vary from mammal to mammal and virus to virus. Those of skill in the art are well equipped by conventional protocols, given the identification of targets and compounds herein, to identify specific dosages for specific

15   mammals, specific viruses and specific modes of administration

METHODS OF USING ANTI-TSG101 ANTIBODIES FOR DELIVERING THERAPEUTIC AND/OR DIAGNOSTIC AGENTS

The invention provides methods and compositions for using anti-TSG101 antibodies for delivering therapeutic and/or diagnostic agents to viral infected cells.

20   Infected cells can be targeted and killed using anti-TSG101 antibody-drug conjugates. For example, an anti-TSG101 antibody may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, or a radioactive metal ion. Antibody-drug conjugates can be prepared by method known in the art (see, e.g., Immunoconjugates, Vogel, ed. 1987; Targeted Drugs, Goldberg, ed. 1983; Antibody Mediated Delivery Systems, Rodwell, ed.

25   1988). Therapeutic drugs, such as but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine,

colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof, can be conjugated to anti-TSG101 antibodies of the invention. Other therapeutic agents that can be conjugated to anti-TSG101

5   antibodies of the invention include, but are not limited to, antimetabolites, e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine; alkylating agents, e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin; anthracyclines, e.g., daunorubicin

10   (daunomycin) and doxorubicin; antibiotics, e.g., dactinomycin (actinomycin), bleomycin, mithramycin, anthramycin (AMC); and anti-mitotic agents, e.g., vincristine and vinblastine. The therapeutic agents that can be conjugated to anti-TSG101 antibodies of the invention may also be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin.

15   The drug molecules can be linked to the anti-TSG101 antibody via a linker. Any suitable linker can be used for the preparation of such conjugates. In some embodiments, the linker can be a linker that allows the drug molecules to be released from the conjugates in unmodified form at the target site.

          The antibodies can also be used diagnostically to, for example, monitor the progression

20   of a viral infection as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive

25   paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of

suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or

acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin

and avidin/biotin; examples of suitable fluorescent materials include fluorescent proteins, e.g.,

green fluorescent protein (GFP), umbelliferone, fluorescein, fluorescein isothiocyanate,

5   rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of

a luminescent material includes luminol; examples of bioluminescent materials include

luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I,

^{131}I, ^{111}In ^{177}Lu, ^{90}Y or ^{99}Tc.

Techniques for conjugating therapeutic moieties to antibodies are well known, see, e.g.,

10   Arnon, et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in

Monoclonal Antibodies And Cancer Therapy, Reisfeld, et al. (eds.), pp. 243-56 (Alan R. Liss,

Inc. 1985); Hellstrom, et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd

Ed.), Robinson, et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers

Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies `84: Biological

15   And Clinical Applications, Pinchera, et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And

Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in

Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin, et al. (eds.), pp. 303-16

(Academic Press 1985), and Thorpe, et al., "The Preparation And Cytotoxic Properties Of

Antibody-Toxin Conjugates", Immunol. Rev., 62,119-58 (1982); each of which is incorporated

20   herein by reference.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody

heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated

herein by reference.

DETECTION OF VIRAL INFECTED CELLS

Antibodies or labeled antibodies directed against a Tsgl01 protein, e.g., an N-terminal region or a C-terminal region of a TSG101 protein, may also be used as diagnostics and prognostics of viral infection, e.g., by detecting the presence of TSG101 protein on cell surface. Such diagnostic methods, may also be used to detect abnormalities in the level of Tsg101 gene

5   expression, or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of a Tsg101 protein.

The tissue or cell type to be analyzed may include those which are known, or suspected, to be infected by a virus. The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A

10   Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cell taken from culture may be a necessary step in the assessment of cells to be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the TSGl01 gene.

15   Preferred diagnostic methods for the detection of TSG101 fragments or conserved variants or peptide fragments thereof, may involve, for example, immunoassays wherein the TSG101 fragments or conserved variants or peptide fragments are detected by their interaction with an anti-TSG101 fragment-specific antibody.

For example, antibodies, or fragments of antibodies, such as those described above useful

20   in the present invention may be used to quantitatively or qualitatively detect infected cells by the presence of TSG101 fragments or conserved variants or peptide fragments thereof on their surfaces. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody (see below, this Section) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially useful in viral infection

25   where TSG101 fragments are recruited to the cell surface during the viral budding process.

The antibodies (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of TSG101 fragments or conserved variants or peptide fragments thereof. *In situ* detection may be accomplished by removing a histological specimen from a patient, and

5   applying thereto a labeled antibody of the present invention. The antibody (or fragment) is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the TSG101 fragment, or conserved variants or peptide fragments, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that

10   any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Immunoassays for TSG101 fragments or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a

15   detectably labeled antibody capable of identifying TSG101 fragments or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of

20   immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled Tsg101 protein specific antibody. The solid phase support may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on solid support may then be detected by conventional means.

25   By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene,

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polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tub, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of anti-TSG101 fragment antibody may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

One of the ways in which the TSG101 gene peptide-specific antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, DiagnosticHorizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller, A., et al., 1978, J. Clin. Pathol. 31:507-520; Butler, J.E., 1981, Meth. Enzymol. 73:482-523; Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,; Ishikawa, E., et al., (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, betagalactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase,

glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

5   Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect TSG101 peptides through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein).

10   The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography.

           It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling

15   compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

           The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or

20   ethylenediaminetetraacetic acid (EDTA).

           The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol,

25   theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

DEPLETION OF VIRAL INFECTED CELLS IN VITRO

The invention provides methods of depleting viral infected cells from non infected tissues and/or cells *in vitro* (or *ex vivo)*. For example, the tissue obtained from a mammal for the *in vitro* depletion of viral infected cells from non infected cells can be blood or serum or other body fluid. In particular, the invention provides for methods of depleting viral infected cells by killing them or by separating them from non infected cells. In one embodiment, anti-TSG101 antibodies are combined, e.g., incubated, *in vitro* with tissues and/or cells obtained from a mammal, e.g., a human.

In one embodiment, a column containing a TSG101 antibody, e.g., an antibody that binds the N-Terminal or C-terminal region of a TSG101 protein, bound to a solid matrix is used to remove viral infected cells from a biological sample, e.g., blood or serum or other body fluid.

The anti-TSG101 antibodies used in the *in vitro* depletion of viral infected cells from tissues can be conjugated to detectable labels (e.g., various enzymes, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials) or therapeutic agents (e.g., cytostatic and cytocidal agents), which are disclosed in section Methods of Using Anti-SG101 Antibodies for Inhibiting Viral Release.

Anti-TSG101 antibodies conjugated to detectable substances can be utilized to sort viral infected cells from non infected cells by methods known to those of skill in the art. In one embodiment, viral infected cells are sorted using a fluorescence activated cell sorter (FACS).

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Fluorescence activated cell sorting (FACS) is a well-known method for separating particles, including cells, based on the fluorescent properties of the particles (Kamarch, 1987, Methods Enzymol., 151:150-165). Laser excitation of fluorescent moieties in the individual particles results in a small electrical charge allowing electromagnetic separation of positive and negative particles from a mixture.

In one embodiment, cells, e.g, blood cells, obtained a mammal, e.g., a human, are incubated with fluorescently labeled TSG101 specific antibodies for a time sufficient to allow the labeled antibodies to bind to the cells. In an alternative embodiment, such cells are incubated with TSG101 specific antibodies, the cells are washed, and the cells are incubated with a second labeled antibody that recognizes the TSG101 specific antibodies. In accordance with these embodiments, the cells are washed and processed through the cell sorter, allowing separation of cells that bind both antibodies to be separated from hybrid cells that do not bind both antibodies. FACS sorted particles may be directly deposited into individual wells of 96-well or 384-well plates to facilitate separation.

In another embodiment, magnetic beads can be used to separate viral infected cells from non infected cells. Viral infected cells may be sorted using a magnetic activated cell sorting (MACS) technique, a method for separating particles based on their ability to bind magnetic beads (0.5-100 nm diameter) (Dynal, 1995). A variety of useful modifications can be performed on the magnetic microspheres, including covalent addition of antibody which immunospecifically recognizes TSG101. A magnetic field is then applied, to physically manipulate the selected beads. The beads are then mixed with the cells to allow binding. Cells are then passed through a magnetic field to separate out viral infected cells.

DOSE OF ANTI-TSG101 ANTIBODIES

The dose can be determined by a physician upon conducting routine tests. Prior to administration to humans, the efficacy is preferably shown in animal models. Any animal model for an infectious disease known in the art can be used.

In general, for antibodies, the preferred dosage is 0.1 mg/kg to 100 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration are often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank, et al., 1997, J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193.

As defined herein, a therapeutically effective amount of anti-TSG101 antibody (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of an anti-TSG101 antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with an anti-TSG101 antibody in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of an anti-TSG101 antibody, used for treatment may increase or decrease over the course of a

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particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

It is understood that appropriate doses of anti-TSG101 antibody agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the anti-TSG101 antibody will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the anti-TSG101 antibody to have upon an infectious agent.

PHARMACEUTICAL FORMULATION AND ADMINISTRATION

The anti-TSG101 antibodies of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise anti-TSG101 antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the anti-TSG101 antibody, use thereof in the compositions is contemplated. Supplementary anti-TSG101 antibodies can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Preferred routes of administration include subcutaneous and intravenous. Other examples of routes of administration include parenteral, intradermal, transdermal (topical), and transmucosal. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene

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glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or

5   bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposablesyringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers

10   include physiological saline, bacteriostatic water, Cremophor EL^{TM} (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that the viscosity is low and the anti-TSG101 antibody is injectable. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

15   The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be

20   achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum

25   monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the anti-TSG101 antibody (e.g., one or more anti-TSG101 antibodies) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the anti-TSG101 antibody

5   into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

10   In one embodiment, the anti-TSG101 antibodies are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of

15   such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811

20   which is incorporated herein by reference in its entirety.

It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of anti-TSG101 antibody calculated to produce the desired therapeutic

25   effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics

of the anti-TSG101 antibody and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an anti-TSG101 antibody for the treatment of individuals.

The pharmaceutical compositions can be included in a kit, in a container, pack, or
5   dispenser together with instructions for administration.

TSG101 VACCINES AND DNA VACCINES FOR TREATMENT AND PREVENTION OF VIRAL INFECTION

The invention provides fragments of a TSG101 protein which can be used as vaccines to generate anti-TSG101 antibodies. The TSG101 protein fragment or polypeptide can be prepared
10   by standard method known in the art. In one embodiment, the invention provides a fragment of a TSG101 protein not comprising the UEV domain of a TSG101 protein. In a specific embodiment, the invention provides a fragment of a human TSG101 protein, or its murine homolog, not comprising the UEV domain. In a preferred embodiment, the invention provides a fragment comprising the C-terminal region of a TSG101 protein. In another embodiment, the
15   invention provides a fragment of a TSG101 protein comprising the coiled-coil domain of a TSG101 protein. In still another embodiment, the invention provides a fragment of a TSG101 protein comprising C-terminaldomain of a TSG101 protein as described SEQ ID NO:3. The invention also provides any sequence that is at least 30%, 50%, 70%, 90%, or 95% homologous such fragments of a TSG101 protein. In some embodiments of the invention, the TSG101
20   protein fragments or polypeptides are at least 5, 10, 20, 50, 100 amino acids in length.

The invention also provides fragment of a TSG101 protein which is functionally equivalent to any TSG101 fragment described above. Such an equivalent TSG101 fragment may contain deletions, additions or substitutions of amino acid residues within the amino acid sequence encoded by the TSG101 protein gene sequences encoding the TSG101 protein but
25   which result in a silent change, thus producing a functionally equivalent TSG101 protein

50

fragment. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature

of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral

5   amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Functionally equivalent", as utilized herein, refers to a protein fragment capable of exhibiting a substantially similar *in vivo* activity as the endogenous TSG101 protein fragment.

10   The TSG101 peptide fragments of the invention may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the TSG101 polypeptides and peptides of the invention by expressing nucleic acid containing TSG101 gene sequences encoding the TSG101 polypeptide or peptide. Methods which are well known to those skilled in the art can be used to construct expression vectors containing TSG101

15   polypeptide coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, supra*,* and Ausubel, et al., 1989, supra. Alternatively, RNA capable of encoding TSG101 polypeptide sequences maybe chemically synthesized using, for example,

20   synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, M.J. ed., IRL Press, Oxford, which is incorporated herein by reference in its entirety.

The TSG101 peptide can be used in combination with a suitable carrier and/or adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent. An oil/surfactant based adjuvant comprising one or more surfactants combined with one or more

25   non-metabolizable mineral oil or metabolizable oil, such as the Incomplete Seppic Adjuvant (Seppic, Paris, France), may be used. An Incomplete Seppic Adjuvant has comparable effect as

Incomplete Freund's Adjuvant for antibody production, but induces lower inflammatory response.

The invention also provides portions of a TSG101 gene for use as DNA or RNA vaccine. The TSG101 gene fragments can also be used for producing any TSG101 protein fragment of the invention described above. In a preferred embodiment, the invention provides a fragment of a TSG101 gene comprising the nucleotide region encoding a fragment not comprising the UEV domain of a TSG101 protein. In a specific embodiment, the fragment of a TSG101 gene is a fragment of a human TSG101 gene, or its murine homolog. The invention also provides any sequence that is at least 30%, 50%, 70%, 90%, or 95% homologous to such fragments of a TSG101 gene. In some embodiments of the invention, the fragment of a TSG101 gene is at least 20, 25, 40, 60, 80, 100, 500, 1000 bases in length. Such sequences may be useful for production of TSG101 peptides.

The invention also provides (a) DNA vectors that contain any of the foregoing TSG101 coding sequences and/or their complements (i.e., antisense); (b) DNA expression vectors that contain any of the foregoing TSG101 coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences; and (c) genetically engineered host cells that contain any of the foregoing TSG 101 coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell for use in producing a TSG101 protein fragment of the invention. As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast a-mating factors.

In another embodiment, the present invention provides a naked DNA or RNA vaccine, and uses thereof. The TSG101 DNA fragment of the present invention described above can be administered as a vaccine to inhibit viral disease by eliciting anti-TSG101 antibodies of the invention. The DNA can be converted to RNA for example by subcloning the DNA into a

5   transcriptional vector, such as pGEM family of plasmid vectors, or under control of a transcriptional. promoter of a virus such as vaccinia, and the RNA used as a naked RNA vaccine. The naked DNA or RNA vaccine can be injected alone, or combined with one or more DNA or RNA vaccines directed to the virus.

The naked DNA or RNA vaccine of the present invention can be administered for

10   example intermuscularly, or alternatively, can be used in nose drops. The DNA or RNA fragment or a portion thereof can be injected as naked DNA or RNA, as DNA or RNA encapsulated in liposomes, as DNA or RNA entrapped in proteoliposomes containing viral envelope receptor proteins (Nicolau, C. et al. Proc. Natl. Acad. Sci. U.S.A. 1983, 80, 1068; Kanoda, Y., et al. Science 1989, 243, 375; Mannino, R. J., et al. Biotechniques 1988, 6, 682).

15   Alternatively, the DNA can be injected along with a carrier. A carrier can be a protein or such as a cytokine, for example interleukin 2, or a polylysine-glycoprotein carrier (Wu, G. Y. and Wu, C. H., J. Biol. Chem. 1988, 263, 14621), or a nonreplicating vector, for example expression vectors containing either the Rous sarcoma virus or cytomegalovirus promoters. Such carrier proteins and vectors and methods for using same are known to a person in the art (See for

20   example, Acsadi, G., et al. Nature 1991, 352, 815-818). In addition, the DNA or RNA could be coated onto tiny gold beads and the beads introduced into the skin with, for example, a gene gun (Cohen, J. Science 1993, 259, 1691-1692; Ulmer, J. B., et al. Science 1993, 259, 1745-1749).

The invention also provides methods for treating a viral infection, e.g., HIV infection, in an animal by gene therapy. A variety of gene therapy approaches may be used to introduce

25   nucleic acid encoding a fragment of the TSG101 protein *in vivo* into cells so as to produce TSG101 antibodies.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below. For general reviews of the methods of gene therapy, see Goldspiel, et al., 1993, Clinical Pharmacy 12, 488-505; Wu and Wu, 1991, Biotherapy 3, 87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32, 573-596; Mulligan,

5   1993, Science 260, 926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62, 191-217; May, 1993, TIBTECH 11, 155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel, et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, New York; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, New York.

10   In a preferred aspect, the therapeutic comprises a TSG101 nucleic acid that is part of an expression vector that expresses a TSG101 or fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the TSG101 coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the TSG101 coding sequences

15   and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the TSG101 nucleic acid (see e.g., Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86, 8932-8935; Zijlstra, et al., 1989, Nature 342, 435-438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient

20   is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro,* then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous

25   methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a

defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by

direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun;

Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents,

encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to

5   a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject

to receptor-mediated endocytosis (see e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432)

(which can be used to target cell types specifically expressing the receptors), etc. In another

embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a

fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal

10   degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific

uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO

92/06180 dated April 16, 1992 (Wu, et al.); WO 92/22635 dated December 23, 1992 (Wilson, et

al.); WO92/20316 dated November 26, 1992 (Findeis, et al.); WO93/14188 dated July 22, 1993

(Clarke, et al.), WO 93/20221 dated October 14, 1993 (Young)). Alternatively, the nucleic acid

15   can be introduced intracellularly and incorporated within host cell DNA for expression, by

homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86,

8932-8935; Zijlstra, et al., 1989, Nature 342, 435-438).

In a specific embodiment, a viral vector that contains the TSG101 nucleic acid is used.

For example, a retroviral vector can be used (see Miller, et al., 1993, Meth. Enzymol.

20   217:581-599). These retroviral vectors have been modified to delete retroviral sequences that

are not necessary for packaging of the viral genome and integration into host cell DNA. The

TSG101 nucleic acid to be used in gene therapy is cloned into the vector, which facilitates

delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen,

et al., 1994, Biotherapy 6, 291-302, which describes the use of a retroviral vector to deliver the

25   mdrl gene to hematopoietic stem cells in order to make the stem cells more resistant to

chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are:

Clowes, et al., 1994, J. Clin. Invest. 93, 644-651; Kiem, et al., 1994, Blood 83, 1467-1473;

Salmons and Gunzberg, 1993, Human Gene Therapy 4, 129-141; and Grossman and Wilson, 1993, Curr. Opin. Genet. and Devel. 3, 110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses

5   naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson (1993, Current Opinion in Genetics and Development 3, 499-503) present a review of adenovirus-based gene therapy. Bout, et al. (1994, Human Gene Therapy 5, 3-10)

10   demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld, et al., 1991, Science 252, 431-434; Rosenfeld, et al., 1992, Cell 68, 143-155; and Mastrangeli, et al., 1993, J. Clin. Invest. 91, 225-234.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh, et

15   al., 1993, Proc. Soc. Exp. Biol. Med. 204, 289-300).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are

20   expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion,

25   chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc.

Numerous techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr, 1993, Meth. Enzymol. 217, 599-618; Cohen, et al., 1993, Meth. Enzymol. 217, 618-644; Cline, 1985, Pharmac.Ther. 29, 69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, epithelial cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled person in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, a TSGl01 nucleic acid is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and

maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention. Such stem cells include but are not limited to hematopoietic stem cells (HSC), stem cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (PCT Publication WO 94/08598), and neural stem cells (Stemple and Anderson,

5   1992, Cell 71, 973-985).

Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures (Rheinwald, 1980, Meth. Cell Bio. 21A:229). In stratified epithelial tissue such as the skin, renewal occurs by mitosis of stem cells within the germinal layer, the layer closest to the basal lamina. Stem cells within the lining of the gut

10   provide for a rapid renewal rate of this tissue. ESCs or keratinocytes obtained from the skin or lining of the gut of a patient or donor can be grown in tissue culture (Rheinwald, 1980, Meth. Cell Bio. 21A, 229; Pittelkow and Scott, 1986, Mayo Clinic Proc. 61, 771). If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (e.g., irradiation, drug or antibody administration to promote moderate immunosuppression) can also be used.

15   With respect to hematopoietic stem cells (HSC), any technique which provides for the isolation, propagation, and maintenance *in vitro* of HSC can be used in this embodiment of the invention. Techniques by which this may be accomplished include (a) the isolation and establishment of HSC cultures from bone marrow cells isolated from the future host, or a donor, or (b) the use of previously established long-term HSC cultures, which may be allogeneic or

20   xenogeneic. Non-autologous HSC are used preferably in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. In a particular embodiment of the present invention, human bone marrow cells can be obtained from the posterior iliac crest by needle aspiration (see e.g., Kodo, et al., 1984, J. Clin. Invest. 73, 1377-1384). In a preferred embodiment of the present invention, the HSCs can be made highly

25   enriched or in substantially pure form. This enrichment can be accomplished before, during, or after long-term culturing, and can be done by any techniques known in the art. Long-term

cultures of bone marrow cells can be established and maintained by using, for example, modified Dexter cell culture techniques (Dexter, et al., 1977, J. Cell Physiol. 91, 335) or Witlock-Witte culture techniques (Witlock and Witte, 1982, Proc. Natl. Acad. Sci. USA 79, 3608-3612).

5   In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Additional methods that can be adapted for use to deliver a nucleic acid encoding a TSG101 fragment of the invention or functional derivative thereof are described below.

BROAD APPLICABILITY OF TSG101 ANTIBODIES TO TREATMENT OF MANY VIRUS
AND VIRUS RELATED DISEASES

Using TSG101 antibodies, we have demonstrated that antibody targeting of TSG101 can
directly inhibit viral infection independent of innate host defense mechanisms (ADCC or CDC).
This finding is consistent with evidence that TSG101 interactions with viral late domain proteins
are essential for the propagation of many different viruses. These results lead us to the
conclusion that many or all of the TSG101 antibody candidates of this invention could have the
ability to directly inhibit viral infection by blocking critical interactions.

Based on the known involvement of TSG101 in many different viral infections, the
activity reported herein is anticipated to be relevant to infections and diseases caused by multiple
and different viruses. A partial listing of these viruses can be found in Figure XX and involves
virtually all known viral Groupings.

Viral Groupings:

- **Group I**: viruses possess double-stranded DNA and include such virus families as
  Herpesviridae (examples like HSV1 (oral herpes), HSV2 (genital herpes), VZV
  (chickenpox), EBV (Epstein-Barr virus), CMV (Cytomegalovirus)), Poxviridae
  (smallpox) and many tailed bacteriophages. The mimivirus was also placed into this
  group.

- **Group II**: viruses possess single-stranded DNA and include such virus families as
  Parvoviridae and the important bacteriophage M13.

Virus Family	Virus Genus	Virion-naked/ enveloped	Capsid Symmetry	Type of nucleic acid
1.Adenoviridae	Adenovirus	Naked	Icosahedral	ds
2.Papovaviridae	Papillomavirus	Naked	Icosahedral	ds circular
3.Parvoviridae	B 19 virus	Naked	Icosahedral	ss
4.Herpesviridae	Herpes Simplex Virus, Varicella zoster virus, Cytomegalovirus, Epstein Barr virus	Enveloped	Icosahedral	ds
5.Poxviridae	Small pox virus, Vaccinia virus	Complex coats	Complex	ds
6.Hepadnaviridae	Hepatitis B virus	Enveloped	Icosahedral	ds circular
7.Polyomaviridae	Polyoma virus (progressive multifocal leucoencephalopathy)	?	?	ds

RNA viruses

- **Group III**: viruses possess double-stranded RNA genomes, e.g. rotavirus. These genomes are always segmented.

- **Group IV**: viruses possess positive-sense single-stranded RNA genomes. Many well known viruses are found in this group, including the picornaviruses (which is a family of viruses that includes well-known viruses like Hepatitis A virus, enteroviruses, rhinoviruses, poliovirus, and foot-and-mouth virus), SARS virus, hepatitis C virus, yellow fever virus, and rubella virus.

- **Group V**: viruses possess negative-sense single-stranded RNA genomes. The deadly Ebola and Marburg viruses are well known members of this group, along with influenza virus, measles, mumps and rabies.

Virus Family	Virus Genera	Virion-naked/ enveloped	Capsid Symmetry	Type of nucleic acid
1.Reoviridae	Reovirus, Rotavirus	Naked	Icosahedral	ds
2.Picornaviridae	Enterovirus, Rhinovirus, Hepatovirus, Cardiovirus, Aphthovirus, Parechovirus, Erbovirus, Kobuvirus, Teschovirus	Naked	Icosahedral	ss
3.Caliciviridae	Norwalk virus, Hepatitis E virus	Naked	Icosahedral	ss
4.Togaviridae	Rubella virus	Enveloped	Icosahedral	ss
5.Arenaviridae	Lymphocytic choriomeningitis virus	Enveloped	Complex	ss

6.Retroviridae	HIV-1, HIV-2, HTLV-I	Enveloped	Complex	ss
7.Flaviviridae	Dengue virus, Hepatitis C virus, Yellow fever virus	Enveloped	Complex	ss
8.Orthomyxoviridae	Influenzavirus A, Influenzavirus B, Influenzavirus C, Isavirus, Thogotovirus	Enveloped	Helical	ss
9.Paramyxoviridae	Measles virus, Mumps virus, Respiratory syncytial virus	Enveloped	Helical	ss
10.Bunyaviridae	California encephalitis virus, Hantavirus	Enveloped	Helical	ss
11.Rhabdoviridae	Rabies virus	Enveloped	Helical	ss
12.Filoviridae	Ebola virus, Marburg virus	Enveloped	Helical	ss
13.Coronaviridae	Corona virus	Enveloped	Complex	ss
14.Astroviridae	Astrovirus	Naked	Icosahedral	ss
15.Bornaviridae	Borna disease virus	Enveloped	Helical	ss

Reverse transcribing viruses

- **Group VI**: viruses possess single-stranded RNA genomes and replicate using reverse transcriptase. The retroviruses are included in this group, of which HIV is a member.

- **Group VII**: viruses possess double-stranded DNA genomes and replicate using reverse
5   transcriptase. The hepatitis B virus can be found in this group.

We have discovered that many of TSG101 is necessary for the propagation of many different viruses and is highly conserved among mammalian or eukaryotic species. Consequently, these compounds could have application for human or veterinary viral diseases. These viral diseases could include but are not limited to PRRS virus, porcine or bovine circoviruses, porcine or bovine

10   coronaviruses, porcine or bovine RSV, porcine, bovine or avian influenza, EIAV, bluetongue, or foot and mouth diseases (FMD) viruses.

Some viruses are causative of more chronic diseases and the morbidity or mortality relates to the presence of virus. These diseases include hepatocellular carcinoma (associated with either HBV or HCV), chronic fatigue syndrome (associated with EBV) and other diseases linked with viral

15   infection.

The compounds above could be used for the treatment or prevention (prophylaxis) of single viral pathogens (e.g., HIV or HBV) or combinations thereof (HIV and HBV). Likewise, these individual or broad-spectrum applications could entail any or all of the virus groups detailed above.

Another method could be the use of the compounds for certain indications associated with

20   one or more viruses. For example, these antibodies could be used for the prevention or treatment of respiratory virus infections, which can be caused by one or more of the pathogens from the groups identified above. As shown in Figure 32, the anti-TSG101 antibodies of the invention bind to RSV infected cells. The administration of this agent to an individual in need of RSV therapy

simultaneously provides prophylaxis against opportunistic influenza, a typical combination of viral agents. Likewise, these compounds could have application against one or more blood-borne pathogens (e.g., HIV and/or HBV and HCV).

The compounds could have application for the prevention, treatment or maintenance of acute or chronic viruses. Acute applications include short-term prevention or treatment of viral infection, examples of which include influenza, rotavirus or filovirus infection. Chronic applications could include recurrent outbreaks, such as is observed with genital herpes) or infrequent outbreaks (such as those associated with zoster infection during shingles). Likewise, treatment could be intended over the long term to maintain low levels of viral load for chronic virus infection (e.g., for HIV, HBV or HCV treatment).

EXAMPLES

EXAMPLE 1: PREPARATION AND USES OF ANTI-TSG101 POLYCLONAL ANTIBODIES

To determine the effect of anti-TSG101 antibodies on viral infections, a retroviral infection assay was developed. A murine leukemia virus (MLV) derived vector which contains an *E. coli lacZ* gene expressed from the long terminal repeat (LTR) promoter (pBMN-Z-I-Neo) was transfected into an amphotropic murine leukemia retroviral packaging cell line derived from 293 cells (Phoenix A, ATCC). Retroviruses produced by the Phoenix A helper cells were collected and used to infect a mouse N2A cells (ATCC). Anti-TSG101 antibodies were added to 293 helper cells 24 hours after the transfection of the MLV vector. The effectiveness of TSG101 antibodies on viral production was determined by the efficiency of viral supernatant to infect the target cells (N2A). The infection of N2A cells was then determined by cellular staining of P-galactosidase activity (positive cells were stained blue, showed as dark spots in Figure 2).

Typically, phoenix A cells were seeded on poly-D-lysine coated 6-well plate a day before transfection. Four microgram of pBMN-Z-I-Neo was then transfected into each well in the presence of 12 ul of Lipofectamine 2000 (Invitrogen). Twenty-four hours post-transfection,

media were replaced with 1 ml/well of fresh media containing trichostatin A (3uM) and 5 or 10 ug of proper anti-TSG101 antibodies. 24 to 48 hours later, viral supernatants were collected, filtered with 0.2 urn filters, and 1 ml of viral supernatant was mixed with 1 ml of fresh media containing polybrene (10 ug/ml), and then used to infect one well of N2a cells. 48 hours

5   post-infection, N2a cells were fixed and stained with X-Gal as described in the 13-Gal staining kit (Invitrogen). Results were documented by digital images.

In the following experiments, two anti-TSG101 antibodies were tested for their effect on viral infection, a rabbit antibody against N-terminal TSG101 protein, and a rabbit antibody against C-terminal TSG101 protein. The anti-N terminal TSG101 antibody was raised using a

10   N-terminal fragment of the human TSG101 protein: VRETVNVITLYKDLKPVL (SEQ ID NO:2). The anti-C terminal TSG101 antibody was raised using a C-terminal fragment of the human TSG101 protein: QLRALMQKARKTAGLSDLY (SEQ ID NO:3). Rabbit IgG was used as non-specific antibody control. More than 10 independent experiments were performed, and representative results are shown in Figure 2. Phoenix helper cells without treatment of antibody

15   (positive control) showed efficient production of retroviruses, and infection of N2A target cells (left top panel); Rabbit IgG had no effect (left middle panel). The rabbit antibody against N-terminal TSG101 showed about 20%-60% inhibition (left bottom panel). But the rabbit antibody against C-terminal TSG101 significantly inhibited the production of retroviruses, and infection of N2A target cells (50-70% inhibition, right top panel). A mixture of the anti-C

20   terminal and anti-N terminal antibodies gave similar results as the anti-C terminal antibody alone (Right middle panel). N2a cells that were not infected by viruses only showed minimal background staining (right bottom panel). Similar results were also obtained in HIV viral infection assays.

EXAMPLE 2: TSG101 LOCALIZED ON CELL SURFACE DURING VIRAL BUDDING

25   This example shows that domains of TSG101 are exposed on cell surface during HIV release, and anti-TSG101 antibodies inhibited HIV release and infection.

TSG101 localization during viral release

       To demonstrate TSG101 is actively involved viral release at plasma membrane, an expression vector of GFP-TSG 101 fusion protein was constructed and transfected into Phoenix cells (a retroviral helper cell line developed by Nolan et al of Stanford university) that was actively producing M-MuLV viruses. 24 hours after transfection, GFP-TSG101 fusion protein traffic was observed under confocal microscope (Ultraview, Perkin-Elmer). Figures 3A-E show a time course of images of GFP-TSG101 protein translocation from cytoplasm onto cell surface, and then "budding" out of the viral producing cells.

Cell Surface Localization of TSG101 during HIV Budding

       To determine if TSG101 is also actively involved in HIV budding, anti-TSG101 antibodies were used to directly detect cell surface TSG101 in a human CD4+ human T cell line H9 transfected with HIV (designated as H9ABg1), and the untransfected H9 cells were used as control. The two rabbit anti-TSG101 polyclonal antibodies, one against N-terminal (designated as anti-TSG101 "C") and one against C-terminal TSG101 (designated as anti-TSG101 "E"), were used for this study. Both antibodies have been well characterized (Li, et al., 2001, Proc. Natl. Acad. Sci. USA 98(4): 1619-24). Both antibodies specifically detected cell surface localization of TSG101 only in HIV producing H9ΔBgl cells, and no cell surface TSG101 was detected in control H9 cells(Figure 4). Interestingly, anti-TSG101 antibodies detected a "capping" like budding structure as observed with anti-HIV antibodies (Lee, et al.,1999, J. Virol. 73, 5654-62).

FACS Profile of Cell Surface Localization of TSG101 during HIV Budding

Cell surface localization of TSG101 in HIV producing cells (H9ΔBgl) and control H9 cells was then examined by Fluorescence Activated Cell Sorter (FACS). Both H9ΔBg1 and H9 cells were fixed, stained with anti-TSG101 antibodies, and detected with a fluorescence labeled secondary antibody. The immuno-stained cells were analyzed on FACS. More than six independent experiments showed that about 70-85% H9ΔBg1 cells were stained positive for surface TSG101, while less than about 0.1% H9 control cells were stained positive for surface TSG101 (Figure 5). These results were further confirmed by direct examination of both H9ΔBg1 and H9 control cells under confocal microscope. The small population (less than 0.1%) of H9 control cells resulted from weak background fluorescence signals associated with immunostaining procedure after confocal microscope analysis. The positive population of H9ΔBg1 cells showed bright fluorescence.

Anti-TSG101 polyclonal antibody inhibition of HIV production in transfected 293 cells

A HIV-1 viral production assay was used to further examine the inhibitory effect of TSG101 on retroviral production. The HIV-1 vector pNL4-3 was transfected into 293T cells. 24 hours after transfaction, two anti-TSG101 antibodies (10 ug/ml), anti-TSG101 antibody "E" and anti-TSG101 antibody "B", and a non-specific control antibody (10 ug/ml) were added respectively into the cell cultures. Anti-TSG101 antibody "B" was raise against a murine TSG101 N-terminal fragment and binds poorly to human TSG101 protein. Anti-TSG101 antibody "B" was used as a control. After an additional 24 hours incubation, cell lysates were extracted, cell culture supernatants were collected and HIV-1 virions were purified by sucrose gradients. Both cell lysates and purified virions were analyzed by Western blot using two anti-HIV-1 antibodies (anti-p55 and anti-p24). As shown in Figure 6, anti-TSG101 antibody "E" treatment showed significant inhibition of HIV-1 virion release (more than 70% inhibition by

68

density tracing of the Western blots, Lane 8), while anti-TSG101 antibody "B" (Lane 7) and the control antibody (Lane 6) did not show significant inhibition of HIV-1 release.

Antibody Inhibition of HIV Release from Human CD4+ T Lymphocytes (H90Bgl cells)

To specifically examine the effect of an antibody on HIV release, a HIV release assay based on H9ΔBg1 cells was developed. H9ΔBgl cells are human CD4+ T lymphocytes transfected with an envelop-defective HIV construct (deletion of a Bgl II fragment of HIV genome). The stably transfected H9ΔBg1 cells produce a non-infectious form of HIV (due to the defective HIV envelop, hence cannot infect other H9ΔBg1 cells in the culture), HIV release from H9ΔBgl cells can be directly measured by HIV p24 ELISA of cell culture supernatant. Several concentrations of TSG101 antibody "E" and control antibody (rabbit IgG at the same concentrations) were used to incubate with H9ΔBgl cells. 48 hours after antibody addition, culture supernatants were collected for HIV p24 ELISA.

Significant antibody inhibition of viral release was observed at 80 ug/ml (Figure 7).

Inhibition of HIV infectivity by polyclonal anti-TSG101 antibody

To determine if anti-TSG101 antibody has additional effect on HIV infectivity following viral release, HIV supernatants from Jurkat cells were used to infect MAGI cells in the presence of anti-TSG101 antibody "E" and rabbit IgG as controls (40 ug/ml). Anti-TSG101 antibody showed significant inhibition of HIV infectivity (Figure 8), suggesting TSG101 has a role in HIV maturation and/or infection of target cells following viral release.

EXAMPLE 3: EFFECT OF ANTI-TSG101 MONOCLONAL ANTIBODIES ON HIV INFECTION

Production of anti-TSG101 monoclonal antibodies (mabs)

A peptide consisting of the nineteen amino acids at C-terminal of TSG101

5   (qlralmqkarktaglsdly, SEQ ID NO:3) was synthesized, conjugated with keyhole lympet

hemocyanin (KLH) and used to immunize 5 mice. Sera samples from each mouse were tested by

ELISA against BSA conjugated C-terminal TSG101 peptide. The mouse with the highest

antibody titer was sacrificed and the spleen was fused with myeloma cells to create hybridoma

pools. PE-8 is one of the hybridoma pools that contain two hybridomas that produce mabs

10   recognizing the immunizing peptide (SEQ ID NO:3). Pool PE-8 is also referred to as antibody

pool PE-8.

Anti-TSG101 mabs inhibit wild type and drug-resistant HIV

Briefly, wild-type and drug resistant HIV expression vector were transfected into

HEK293 cells. The transfected cells were treated with different concentration of anti-TSG101

15   antibodies and control antibodies 24 hours post transfection. Following 24- 48 hours incubation

after antibody treatment, cell culture supernatants were collected and used to infected an

indicator cell line (the MAGI cell). The MAGI cells were lysed 24-48 hour post infection and

assayed for luciferase activity. The inhibition of HIV infectivity was determined by reduction of

luciferase activity.   The control antibodies were: N⬜19, which is an mAb against N-terminal of

20   TSG101; and F-1, F-15 and F-19, which are mAbs raised against the full-length TSG101.

As shown in Figure 16, antibody pool PE-8, which targets the 19 amino acid residues at

the C-terminal of TSG101 protein and is in the form of purified IgG from ascites, inhibits the

infectivity of wide type HIV strain NL4-3 in MAGI cells. In contrast, monoclonal antibodies N-

19 and F-1, both are directed to other regions of the TSG101 protein, showed no inhibitory effect

25   on HIV infectivity.

70

Subclones of the PE-8 mab pool were isolated. The supernatant was harvested from each hybridoma clone and was tested for its anti-HIV activities. As shown in Figure 17, the subclones appeared to fall into either a ▢high inhibition▢ group (e.g., H88 and G12) or a ▢low inhibition▢ group (e.g., H10 and F8), which correlates with the fact that the PE-8 pool contains only two

5   mabs. In a representative experiment, the mab produced by the subclone H88 was isolated in the form of purified IgG from ascites and was tested for anti-HIV activity. As shown in Figure 18, the H88 IgG inhibited HIV infection by more than 50%.

As described in more detail in the Background section of the application, current treatment for AIDS utilizes inhibitors for the reverse transcriptase (RT) and protease of HIV.

10   However, despite its initial success in reducing viral load in the AIDS patient, the treatment begins to lose efficacy due to the appearance of drug-resistant HIV strains in newly infected individuals.

In order to determine whether TSG101 is actively involved in the infection process of drug-resistant HIV, dominant negative mutants of TSG101 were generated. Briefly, a TSG101

15   dominate negative mutant expression vector was generated by subcloning TSG101 amino acid residues 1-312 (SEQ ID NO:42) into a expression vector (pLL1), which contains a CMV promoter and polyA signal.

The TSG101 dominate negative expression vector or a control vector was then cotransfected with expression vector for wild-type or drug resistant HIV into HEK293 cells. The

20   culture supernatant were collected 24-48 hours post transfection and were used to infected an indicator cell line (MAGI cells). The MAGI cells were lysed 24-28 hours post infection and assayed for luciferase activity. The inhibition of HIV infectivity was determined by reduction of luciferase activity.

As shown in Figure 19, the dominant negative mutant of TSG101 completely inhibited

25   the infectivity of RT inhibitor resistant HIV strain pL10R and protease inhibitor resistant HIV

strain p1617-1. It thus appears that TSG101 plays an essential role in the infectivity of drug resistant HIV strains.

As demonstrated in Figures 20 and 21, anti-TSG101 mab pool PE-8 also showed significant inhibition on the infectivity of the drug-resistant HIV strains pL10R and p1617-1. In contrast, control mabs F-15 and F-19, both were raised against full-length TSG101, showed no inhibition on the infectivity of the drug-resistant HIV strains. These results suggest that the anti-TSG101 mabs may provide a new treatment for AIDS, especially for those patients infected by drug-resistant HIV strains. Because the anti-TSG101 antibody treatment targets a host protein rather than a viral protein, it would be effective in inhibiting infection by all HIV variants, so long as the TSG101-related pathway is involved in the viral infection. Moreover, since the treatment does not place a direct selection pressure on the viruses, it would slow the development of resistance.

Anti-TSG101 mab inhibits HIV production in human peripheral blood mononuclear cells (PBMC)

<u>Antibody inhibition of HIV infection of Human Peripheral Blood Mononuclear Cells (PBMCs)</u>

To determine the effectiveness of anti-TSG101 antibody inhibition of HIV infection, a human PBMC based HIV infection assay (Pilgrim, et al., J. Infect Dis. 176:924-32, 1997 and Zhou, et al., Virol. 71:2512-7, 1997) was used. Leukocytes were obtained by leukapheresis of HIV-seronegative donors, and PBMCs were isolated by Ficoll-Hypaque gradient centrifugation. Prior to HIV-1 infection, PBMCs were activated by incubation in interleukin-2 (IL-2) cell culture medium containing 10 µg of phytohemagglutinin (PHA) (PHA-P; Difco Laboratories, Detroit, Michigan) per ml. IL-2 culture medium is RPMI 1640 medium containing 100 U of penicillin, 100 µg of streptomycin, 2 mM L-glutamine, 10% heat-inactivated fetal calf serum, and 20 U of recombinant IL-2 (Roche Molecular Biochemicals, Indianapolis, Indiana) per ml.

After overnight incubation with PHA, cells were washed and continued in culture with IL-2 for 3 to 5 days. All cell cultures were maintained in 5% CO2 incubators at 37°C.

HIV-1 isolates were obtained from the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH (including wild-type HIV strain pNL4-3, and drug resistant strains pL10-R and pL1617-1), and were expanded by two or three cycles of growth on PHA- and IL-2-stimulated PBMC. To produce the final virus stock, PBMCs were exposed to undiluted virus for 2 h at a cell concentration of 10^7/ml, and IL-2 culture medium was added to bring the cell concentration to 10^6/ml. IL-2 culture medium was exchanged every 2 days, and supernatants were collected during the peak of HIV p24 expression, usually 5 to 10 days after infection. Virus stocks were made cell free by centrifugation at 1,000 x g and filtration though a 0.45-μm filter. Virus aliquots were stored in the vapor phase of liquid nitrogen. HIV virus 50% tissue culture infectious doses ($TCID_{50}$) were determined by a sensitive 14-day endpoint titration assay using PHA and IL-2-stimulated PBMC as previously described (Mascola, et al., J. Infect Dis. 173:340-8 1996).

HIV-1 infection of PHA- and IL-2-stimulated PBMC was performed in 96-well round-bottomed culture plates by combining 40 μl of virus stock with 20 μl of PBMC (1.5 x 10^5 cells). The multiplicity of infection (MOI) was optimized for individual experiments. Anti-TSG101 antibody inhibition assay was performed in a 96-well plate format. In this assay, 500 to 1,000 $TCID_{50}$ of HIV-1 were added to each well, resulting in an MOI of about 0.01. PBMC are incubation with antibody and virus for 24 hours to 15 days. This assay allows several rounds of virus replication, and therefore virus growth kinetics (measured as extracellular HIV p24 production) is monitored by serial collection of culture supernatants from days 2 to 15. HIV p24 is measured with a commercial ELISA kit (Perkin Elmer). Antibody inhibition of HIV infection was determined by significant inhibition of the production of infectious HIV particles in the cell culture supernatant using a MAGI assay as described by Kimpton, et al. and Wei, et al.

(Kimpton, et al., J. Viol 66, 2232, 1992; Wei, et al., Antimicro Agents Chemother, 46, 1896, 2002). Multiple time points and duration of antibody incubations will be examined for the optimal inhibition of HIV infection of human PBMCs.

As shown in Figures 22 and 23, anti-TSG101 mab pool PE-8 (marked as C8P in the figures) significantly inhibits HIV production in human PBMC after 3-day and 7-day incubation, respectively.

EXAMPLE 4: MOLECULAR CLONING OF ANTI-TSG101 MAB GENES

Genes encoding the mabs in the PE-8 antibody pool (containing two mabs) were cloned. Briefly, The variable regions of the mab genes were amplified by RT-PCR with total RNA from the hybridoma cells, using the degenerate primers designed from the mab leader sequences and the constant domains near the variable region of both chains. The variable domain of the heavy chain for PE-8 pool was amplified with a forward primer (5'-actagtcgacatgtacttgggactgagctgtgtat-3' (SEQ ID NO:4)) and a reverse primer (5'-cccaagcttccagggrccarkggataracigrtgg-3' (SEQ ID NO:5)); while the variable domain of the light chain was amplified with forward primers (a mixture of two degenerate primers 5'-actagtcgacatggtrtccwcasctcagttccttg-3' (SEQ ID NO: 6) and 5'-actagtcgacatgakgthcycigctcagytyctirg-3' (SEQ ID NO: 7)) and a reverse primer (5'-cccaagcttactggatggtgggaagatgga-3' (SEQ ID NO: 8)). The variable domain of the heavy chain of mab3G1 was amplified with the forward primers (a mixture of three degenerate primers 5'-actagtcgacatgatggtgttaagtcttctgtacct-3' (SEQ ID NO:9), 5'-actagtcgacatgaaatgcagctggrtyatsttctt-3' (SEQ ID NO:10), and 5'-actagtcgacatggrcagrcttacwtyytcattcct-3' (SEQ ID NO:11)) and the reverse primer, 5'-cccaagcttccagggrccarkggataracigrtgg-3' (SEQ ID NO:12), while the variable domain of the light chain was amplified with the forward primers (a mixture of four degenerate primers 5'-actagtcgacatgaagttgcctgttaggctgttggtgct-3' (SEQ ID NO:13), 5'-actagtcgacatggatttwcargtgcagattwtcagctt-3' (SEQ ID NO:14), 5'-actagtcgacatggtyctyatvtccttgctgttctgg-3' (SEQ ID NO:15) and 5'-

actagtcgacatggtyctyatvttrctgctgctatgg 3' (SEQ ID NO:16) and a reverse primer, 5'-

cccaagcttactggatggtgggaagatgga-3' (SEQ ID NO:17). The 450-bp PCR products were purified

from the agarose gel and ligated into the TA-cloning vector for sequencing confirmation.

Using the above-described method, the heavy and light chain cDNA sequences of clone

5   D1 (a dominant clone) and clone 3G1 of pool PE-8 were determined. The amplified cDNA

sequences and the variable region nucleic acid and amino acid sequences are summarized in

Table 1.

Table 1: Cloned mab sequences

Gene	Cloned cDNA sequence	Variable region amino acid sequence	Variable region nucleotide sequence
Clone D1 heavy chain (V$_H$)	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20
clone D1 light chain (V$_L$)	SEQ ID NO:21	SEQ ID NO:22	SEQ ID NO:23
clone 3G1 heavy chain (V$_H$)	SEQ ID NO:24	SEQ ID NO:25	SEQ ID NO:26
Clone 3G1 light chain (V$_L$)	SEQ ID NO:27	SEQ ID NO:28	SEQ ID NO:29

10   Figures 23-26 show sequence alignments of the cloned heavy and light chain sequences

to mouse IgG heavy and light chain sequences. The complementarity determining regions

(CDRs) of the mabs are listed in Table 2.

Table 2: Amino acid sequences of the CDRs of mabs D1 and 3G1

CDRs	Amino Acid Sequences
D1 V_H chain CDR2	QIRNKPFNYETFYSDSV (SEQ ID NO:31)
D1 V_H chain CDR3	SNHRYGV (SEQ ID NO:32)
D1 V_L chain CDR2	YSSRLQS (SEQ ID NO:34)
D1 V_L chain CDR3	QQSKAL (SEQ ID NO:35)
3G1 V_H chain CDR2	HIWWDNV (SEQ ID NO:37)
3G1 V_H chain CDR3	ARIEGVNGNYPYF (SEQ ID NO:38)
3G1 V_L chain CDR2	GTSNLAS (SEQ ID NO:40)
3G1 V_L chain CDR3	SSYPLMT (SEQ ID NO:41)

EXAMPLE 5: ANTI-TSG101 ANTIBODIES INHIBIT RELEASE OF EBOLAVIRUS

This Example shows that TSG101 interacts with EBOV VP40, that TSG101 is
incorporated into EBOV VLPs, and that anti-TSG101 antibody inhibits the release of EBOV
virus-like particles (VLPs).

5   The only members of the family Filoviridae, EBOV and MARV possess a
negative-stranded, non-segmented 19 Kb RNA genome comprising 7 genes: nucleoprotein(NP),
viral proteins VP35, VP40, glycoprotein (GP), VP30, VP24, and RNA polymerase(L), encoding
for seven proteins in MARV and eight proteins in EBOV. Recent studies provide some insights
in the cellular localization and role of VP40, a 326 amino acid matrix (M) protein (Jasenosky, et
10   al., 2001, J. Virol. 75 (11): 5205-14; Kolesnikova, et al., 2002, J. Virol 76 (4): 1825-38). In cells
infected with either EBOV or MARV, the majority of VP40 is peripherally associated with the
cytoplasmic face of the plasma membrane via hydrophobic

interactions. Significantly, expression of EBOV and MARV VP40 in transfected cells is
required for the production of virus-like particles (VLPs), non-infectious particles that have
15   some morphological properties similar to authentic viruses. The ability of VP40 to direct its own
release from infected cells was mapped to a proline-rich sequence motif common to other
enveloped RNA viruses (Harty, et al., 2000, Proc. Natl. Acad. Sci. USA 97, 13871-6).

Generation of virus-like particles as a surrogate model for Ebola assembly and release

Several virus-like particles can be generated by mere expression of viral matrix proteins
20   (Johnson, et al., 2000, Curr. Opin. Struct. Biol. 10, 229-235). EBOV and MARV matrix proteins
(VP40) have been shown to localize to both the plasma membrane and viralinclusion bodies
(Kolesnikova, et al., 2002, J. Virol. 76, 1825-38; Martin-Serrano, et al., 2001, Nature Medicine
7, 1313-19), suggesting that VP40 may drive the assembly and release of mature virions.
However, attempts to efficiently generate VLPs by expression of VP40 alone have been largely
25   unfruitful, marked by inefficient release of amorphous VP40-containing material (Bavari, et al.,

2002, J. Exp. Med. 195, 593-602). In retroviruses, the raft localization of the assembly complex

is regulated by the association of N-terminally acylated Gag proteins (Campbell, et al., 2001, J.

Clin. Virol. 22, 217-227), whereas raft targeting of filovirus proteins such as VP40 appear to be

mainly regulated by the viral glycoprotein (GP) (Bavari, et al., 2002, J. Exp. Med. 195,

5   593-602). Therefore, it was hypothesized that generation of filovirus VLPs may require

coexpression of both GP and VP40. Whether GP and VP40 are released into culture

supernatants was first examined. In cells expressing either GP or VP40 alone both proteins

could be detected both in cells and supernatants (Figure 9A). Coexpression of both proteins,

however, resulted in substantial increase in release from cells (Fig 9A). It was reasoned that if

10   the released GP and VP40 are associated in particles, VP40 must be co-immunoprecipitated with

anti-GP mAb. As shown in Figure 9B, VP40 was readily detected in anti

GP-immunoprecipitates from the supernatants of cells transfected with both GP and VP40 of

EBOV. No VP40 was pulled down from the supernatant of cells expressing VP40 alone,

showing that the co-IP is specific.

15   <u>Particles formed by EBOV GP and VP40 display the morphological characteristics of Ebola</u>
<u>virus</u>

The co-IP experiments demonstrated that GP and VP40 released into supernatant are

associated with each other in some form. To determine whether these complexes

representvirus-like particles (VLPs), particulate material from culture supernatants was purified

20   by sucrose gradient ultracentrifugation (Bavari, et al., 2002, J. Exp. Med. 195, 593-602) and

analyzed using electron microscopy. Interestingly, most of the particles obtained from the

supernatants of the cells cotransfected with GP and VP40 displayed a filamentous morphology

strikingly similar to filoviruses (Figures 10A and 10B) (Geisbert, et al.,1995, Virus Res. 39,

129-150; Murphy, et al.,1978, Ebola and Marburg virus morphology and taxonomy, 1 edn

25   (Amsterdam, Elsvier)). In contrast, the material obtained from singly transfected cells only

contained small quantities of membrane fragments, likely released during cell death. The VLPs

have a diameter of 50-70 nm and are 1-2 μm in length (Figure 10). This is similar to the length

range of Ebola virions found in cell culture supernatants after *in vitro* infection (Geisbert, et

al.,1995, Virus Res. 39, 129-150). The smaller diameter of VLPs (as compared to 80 nm for

EBOV) may be due to the lack of ribonucleoprotein complex. All types of morphologies

5   described for filoviruses such as branched, rod-, U-and 6-shaped forms (Feldmann, et al.,1996,

Adv. Virus Res. 47, 1-52; Geisbert, et al.,1995, Virus Res. 39, 129-150) among these particles

were observed (Figure 10). In addition, the VLPs were coated with 5-10 nm surface projections

or "spikes" (Figure 10), characteristic for EBOV (Feldmann, et al.,1996, Adv. Virus Res. 47,

1-52; Geisbert, et al.,1995, Virus Res. 39, 129-150). Immunogold staining of the VLPs with

10   anti-Ebola GP antibodies demonstrated the identity of the spikes on the surface of the particles as

Ebola glycoprotein(Figure 10B). VLPs for Marburg virus were also generated in a similar

manner.

In summary, a surrogate assay for the assembly and release of Ebola virus that can be

performed without the restrictions of biocontainment laboratories was established. This assay

15   can be used for initial screenings of agents that may inhibit Ebola virus budding.

Studies on the role of TSG101 in Ebola virus life cycle

We performed a series of biochemical studies to examine the involvement of the

vaccuolar protein sorting (vps) protein TSG101 interaction with the late domain of VP40 in

EBOV assembly and release. A set of TSG101 truncations C-terminally tagged with a Myc

20   epitope were used for these studies. 293T cells were transfected with full length TSG101 and

mutants truncated at positions 140, 250 and 300 along with EBOV VP40. Cells were lysed after

48h and subjected to immunoprecipitation with an anti-Myc antibody. As shown in Figure 11,

VP40 was coprecipitated with all TSG101 proteins except for the 1-140 truncated mutant. Lack

of association with this mutant is consistent with the structural data that show that residues

25   141-145 make important contacts with an HIV Gag-derived PTAP peptide (Pornillos, et al.,

2002, Nat. Struct. Biol. 9, 812-7). Interestingly, the association of VP40 with 1-300 mutant of

TSG101 was significantly stronger than with full length or 1-250 mutant (Figure 11). The lower association of full length TSG101 can be attributed to the presence of a PTAP motif in the C-terminal region of this molecule that may form an 30 inter- or intramolecular association with the UEV domain of TSG101 . The dramatic reduction of interaction resulting from deletion of

5   amino acids 250-300 suggests that residues in this region may contribute to the binding to viral matrix proteins.

To confirm that TSG101 and VP40 associate directly and through the PTAP motif, Far Western analysis was performed. Ebola VP40 (1-326) and truncated (31-326) Ebola VP40 proteins as well as HA-tagged UEV domain of TSG101 were produced in bacteria. These

10   proteins were electrophoresed on 4-20% gradient gel and electroblotted onto nitrocellulose membrane. Following blocking, the blot was incubated with a purified TSG101 protein (UEV), washed, and protein protein interaction detected by enhanced chemiluminescence using an anti TSG101 antibody and HRP- labeled goat anti rabbit as secondary antibody. As shown in Figure 12, TSG101 interacts with the full length Ebola VP40 but not with the truncated Ebola VP40,

15   confirming that the PTAP motif at the N terminus of VP40 plays a critical role in VP40 TSG101(UEV) interaction. An identical western blot developed with Ebola VP40 antibody could detect both the full length and the truncated VP40 showing the presence of both the proteins on the blot.

Surface plasmon resonance biosensor (SPR) analysis of the Ebola VP40 TSG101 interaction

20   A quantitative analysis of Ebola VP40 interacting with TSG101 was carried out using SPR measurements. A biotinylated peptide (Bio - ILPTAPPEYME, SEQ ID NO:43) containing 11 amino acid residues from the N terminus of the Ebola VP40 was immobilized on the streptavidine chip. Purified TSG101 protein that contains only the UEV domain was injected at different concentrations (1, 2, 5, 20 uM) serially. As seen in the Figure 13, an interaction of

25   moderate affinity between the peptide and proteins can be detected. Based on the SPR data we calculated a Kd value of ~ 2 μM for this interaction.

Incorporation of TSG101 in Ebola VLPs and virions

To determine whether TSG101 is incorporated in EBOV VLPs, TSG101 (1-312, SEQ ID NO:42) together with GP or GP+VP40 were expressed in 293T cells. VLPs were immunoprecipitated from supernatants using anti GP antibodies. Expression of TSG101(1-312)
5 resulted in a marked increase in VLP release, suggesting a positive role for TSG101 in VLP budding (Figure 14A, Lane 4). In addition, TSG101 (1-312) was coimmunoprecipitated with an anti-GP antibody from the culture supernatants when expressed along with GP and VP40 (Figure 14A, Lane 4). No TSG101 was found associated with GP when expressed in the absence of VP40 (Figure 14A, lane 3), suggesting that its association with GP was dependent on formation
10 of VLPs. Similar results were also obtained with full length TSG101. These data strongly suggest that TSG101 is incorporated into VLPs and support the hypothesis that TSG101 plays a role in viral assembly and/or budding. To further substantiate this finding we also analyzed inactivated, band purified, EBOV (iEBOV) for the presence of TSG101. 5μg iEBOV were analyzed by immunoblotting for the presence of TSG101. As shown in Figure 6B, we found
15 readily detectable levels of TSG101 in iEBOV, clearly demonstrating the incorporation of TSG101 in Ebola virus.

Effect of polyclonal anti-TSG101 antibodies on Ebola virus release

The biochemical and VLP release data suggested that TSG101 is critical for the egress of Ebola virus. Therefore, the effects of polyclonal anti-TSG101 antibodies "C" and "E" (that
20 showed inhibitory effect on HIV, see Examples 1 and 2) were tested on the virus production in Hela cells infected with Ebola Zaire-95 virus. Monolayers of Hela cells were incubated with the virus at an MOI of 1 for 50 minutes, washed, and a medium containing an anti-TSG101 antibody or a control rabbit anti mouse antibody were added at 5 μg/ml. After 24 hours the supernatants were harvested and the released viruses enumerated by plaque assay as previously described
25 (Bavari, et al., 2002, J. Exp. Med. 195, 593-602). As shown in Figure 15, these antibodies partially inhibited the release of virions into Hela cell supernatant.

Anti-TSG101 mabs protects mice against EBOV challenge

The anti-TSG101 mabs were also tested for their anti-EBOV activity in mice. Briefly, Balb/c mice in groups of 10 were injected intraperitioneally with either 4mg of the anti-TSG101 mab pool PE-8 (shown as mab C8 in Table 3 and Figure 27) in the form of purified IgG, 4mg of a control mab, or phosphate buffered saline (PBS). One hour after the injection, the mice were challenged with EBOV at a dose of 100 pfu/mouse. As shown in Table 3 and Figure 27, injection of anti-TSG101 mabs significantly increased the survival rate of EBOV challenged mice.

Table 3: Protection of Anti-TSG101 Mab against EBOV

Antibody	Treatment	Survivals / total
PBS control	i.p. on day 0	1 / 10
Mab control	4 mg i.p. on day 0	1 / 10
Mab C8	4 mg i.p. on day 0	5 / 10

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of the present invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific

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embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims along with the full scope of equivalents to which such claims are entitled.

<u>Sequences set forth or recited herein, by SEQ. ID NO. Identification</u>

5　1.

mavsesqlkk mvskykyrdl tvretvnvit lykdlkpvld syvfndgssr elmnltgtip

vpyrgntyni piclwlldty pynppicfvk ptssmtiktg khvdangkiy lpylhewkhp

qsdllgliqv mivvfgdepp vfsrpisasy ppyqatgppn tsympgmpgg ispypsgypp

npsgypgcpy ppggpypatt ssqypsqppv ttvgpsrdgt isedtirasl isavsdklrw

10　rmkeemdraq aelnalkrte edlkkghqkl eemvtrldqe vaevdkniel lkkkdeelss

alekmenqse nndideviip taplykqiln lyaeenaied tifylgealr rgvidldvfl

khvrllsrkq fqlralmqka rktaglsdly

2.

VRETVNVITLYKDLKPVL

15　3.

QLRALMQKARKTAGLSDLY

4.

actagtcgacatgtacttgggactgagctgtgtat

5.

cccaagcttccagggrccarkggataracigrtgg

6.

actagtcgacatggtrtccwcasctcagttccttg

5   7.

actagtcgacatgakgthcycigctcagytyctirg

8.

cccaagcttactggatggtgggaagatgga

9.

10   actagtcgacatgatggtgttaagtcttctgtacct

10.

actagtcgacatgaaatgcagctggrtyatsttctt

11.

actagtcgacatggrcagrcttacwtyytcattcct

15   12.

cccaagcttccagggrccarkggataracigrtgg

13.

actagtcgacatgaagttgcctgttaggctgttggtgct

14.

actagtcgacatggatttwcargtgcagattwtcagctt

15.

actagtcgacatggtyctyatvtccttgctgttctg

5　16.

actagtcgacatggtyctyatvttrctgctgctatgg

17.

cccaagcttactggatggtgggaagatgga

18.

10　ATGTACTTGGGACTGAGCTGTGTATTCATTGTTTTTCTCTTAAAAGGTGTCCAGTGTG
AG

GTGAAGCTGGATGAGACTGGAGGAGGCTTGGTGCAACCTGGGAGGCCCATGAAACT
CTCG

TGTGTTGCCTCTGGATTCACTTTTAGTGACTACTGGATGAACTGGGTCCGCCAGTCTC
15　CA

GAGAAGGGACTGGAGTGGGTAGCGCAAATTAGAAACAAACCGTTTAATTATGAAAC
ATTT

TATTCAGATTCTGTGAAAGGCAGATTCACCATCTCAAGAGATGATTCCAAAAGTAGT
GTC

TACCTGCAAATGAACAACTTAAGAAATGAGGACATGGGTATCTATTACTGTTCAAAT
CAT

AGATATGGGGTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAA
ACG

5   ACACCCCATCCGTTTATCCCTTGGTCCCTGGAAGCTTGGG

19.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPFN
YETFYSDSVKGRFTISRDDSKSSVYLQMNNLRNEDMGIYYCSNHRYGVAYWGQGTLVT
VSA

10   20.

GAGGTGAAGCTGGATGAGACTGGAGGAGGCTTGGTGCAACCTGGGAGGCCCATGAA
ACTCTCG

TGTGTTGCCTCTGGATTCACTTTTAGTGACTACTGGATGAACTGGGTCCGCCAGTCTC
CA

15   GAGAAGGGACTGGAGTGGGTAGCGCAAATTAGAAACAAACCGTTTAATTATGAAAC
ATTT

TATTCAGATTCTGTGAAAGGCAGATTCACCATCTCAAGAGATGATTCCAAAAGTAGT
GTC

TACCTGCAAATGAACAACTTAAGAAATGAGGACATGGGTATCTATTACTGTTCAAAT
20   CAT

AGATATGGGGTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

21.

CNGTCTGTTGCTCTGTTTTCAGGTACCAGATGTGATATCCAGATGACACAGACTACA
ACC

TCCCTGTCTGCCTCTCTGGGAGACAGGGTCACCATCAGTTGCAGGGCAAGTCAGGAC
ATT

AACCATTATTTAAGCTGGTTTCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATC
TTC

TACTCATCAAGATTACAGTCAGGTGTCCCGTCAAGGTTCAGTGGCAGTGGGTCTGGA
AGA

GATTTTTCTCTCACCATTAGGGCCCTGGAACAAGAAGATATTGCCACTTACTTTTGCC
AA

CAAAGTAAAGCGCTCCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACG
GGCT

GATGCTGCACCAACTGTATCCATCTTCCCACCATCCAG

22.

DIQMTQTTTSLSASLGDRVTISCRASQDINHYLSWFQQKPDGTVKLLIFYSSRLQSGVPSR
FSGSGSGRDFSLTIRALEQEDIATYFCQQSKALPWTFGGGTKLEIKR

23.

GATATCCAGATGACACAGACTACAACC

TCCCTGTCTGCCTCTCTGGGAGACAGGGTCACCATCAGTTGCAGGGCAAGTCAGGAC
ATT

AACCATTATTTAAGCTGGTTTCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATC
TTC

TACTCATCAAGATTACAGTCAGGTGTCCCGTCAAGGTTCAGTGGCAGTGGGTCTGGA
AGA

5   GATTTTTCTCTCACCATTAGGGCCCTGGAACAAGAAGATATTGCCACTTACTTTTGCC
AA

CAAAGTAAAGCGCTCCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACG
G

24.

10   GCCCTTACTAGTCGACATGGGCAGGCTTACTTTCTCATTCCTGCTACTGATTGTCCCT
GC

ATATGTCCTGTCCCAGGTTACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCCTC
CCA

GACCCTCAGTCTGACTTGTTCTTTCTCTGGATTCTCACTGAGCACTTTTAATGTGGGT
15   GT

AGGCTGGATTCGTCAGCCATCAGGGAAGGGTCTGGAGTGGCTGGCACACATTTGGT
GGGA

TAATGTCAAGCGCTATAACCCAGCCCTGAAGAGCCGACTGACTATCTCCAAGGATAC
CTC

20   CAGCAGCCAGGTATTCCTCGACATCGCCAGTGTGGACACTGCAGATACTGCCACATA
TTT

88

TTGTGCTCGAATAGAGGGGGTGAATGGTAACTACCCCTACTTTTCTTACTGGGGCCA

AGG

GACTCTGGTCACTGTCTCTGCAGCCAAAACGACACCCCCATCCGTTTATCCCCTGGC

CCC

5   TGGAAGCTTGGG

25.

QVTLKESGPGILQPSQTLSLTCSFSGFSLSTFNVGVGWIRQPSGKGLEWLAHIWWDNVKR

YNPALKSRLTISKDTSSSQVFLDIASVDTADTATYFCARIEGVNGNYPYFSYWGQGTLVT

VSA

10   26.

ATATGTCCTGTCCCAGGTTACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCCTC

CCA

GACCCTCAGTCTGACTTGTTCTTTCTCTGGATTCTCACTGAGCACTTTTAATGTGGGT

GT

15   AGGCTGGATTCGTCAGCCATCAGGGAAGGGTCTGGAGTGGCTGGCACACATTTGGT

GGGA

TAATGTCAAGCGCTATAACCCAGCCCTGAAGAGCCGACTGACTATCTCCAAGGATAC

CTC

CAGCAGCCAGGTATTCCTCGACATCGCCAGTGTGGACACTGCAGATACTGCCACATA

20   TTT

TTGTGCTCGAATAGAGGGGGTGAATGGTAACTACCCCTACTTTTCTTACTGGGGCCA
AGG

GACTCTGGTCACTGTCTCTGCA

27.

5   ACTAGTCGACATGGATTTACAGGTGCAGATTATCAGCTTCATGCTAATCAGTGTCAC
AGT

CATATTGTCCAGTGGAGAAATTGTGGTCACCCAGTCTCCGGCACTCATGGCTGCATC
TCC

AGGGGAGAGGGTCACCATCACCTGCAGTGTCAGCTCAAGTATAAATTCCAACAACTT
10 ACA

CTGGTACCAACAGAAGTCAGAAGCCTCCCCCAAACCCTGGATTTATGGCACATCCAA
CCT

GGCTTCTGGAGTCCCTGTTCGCTTCAGTGGCAGTGGATCTGGGACCTCTTTTTCTCTC
AC

15 AGTCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGTCAACAGTGGAGTA
GTTA

CCCACTCATGACGTTCGGTGGGGGCACCAAACTGGAAATCAAGCGGGCTGATGCTG
CACC

AACTGTATCCATCTTCCACCATCCAGTAAGCTTGGG

20 28.

EIVVTQSPALMAASPGERVTITCSVSSSINSNNLHWYQQKSEASPKPWIYGTSNLASGVP
VRFSGSGSGTSFSLTVSSMEAEDAATYYCQQWSSYPLMTFGGGTKLEIKR

29.

CATATTGTCCAGTGGAGAAATTGTGGTCACCCAGTCTCCGGCACTCATGGCTGCATC
TCC

AGGGGAGAGGGTCACCATCACCTGCAGTGTCAGCTCAAGTATAAATTCCAACAACTT
ACA

CTGGTACCAACAGAAGTCAGAAGCCTCCCCCAAACCCTGGATTTATGGCACATCCAA
CCT

GGCTTCTGGAGTCCCTGTTCGCTTCAGTGGCAGTGGATCTGGGACCTCTTTTTCTCTC
AC

AGTCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGTCAACAGTGGAGTA
GTTA

CCCACTCATGACGTTCGGTGGGGGCACCAAACTGGAAATCAAGCGG

30.

DYWMN

31.

QIRNKPFNYETFYSDSV

32.

SNHRYGV

91

33.

RASQDINHYLS

34.

YSSRLQS

5   35.

QQSKAL

36.

FNVGVG

37.

10   HIWWDNV

38.

ARIEGVNGNYPYF

39.

RVTITCSVSSSINSNNLH

15   40.

TSNLAS

41.

SSYPLMT

42.

mavsesqlkk mvskykyrdl tvretvnvit lykdlkpvld syvfndgssr elmnltgtip

vpyrgntyni piclwlldty pynppicfvk ptssmtiktg khvdangkiy lpylhewkhp

qsdllgliqv mivvfgdepp vfsrpisasy ppyqatgppn tsympgmpgg ispypsgypp

npsgypgcpy ppggpypatt ssqypsqppv ttvgpsrdgt isedtirasl isavsdklrw

rmkeemdraq aelnalkrte edlkkghqkl eemvtrldqe vaevdkniel lkkkdeelss

alekmenqse nn

43.

ILPTAPPEYME

WHAT IS CLAIMED IS:

1. A method for inhibiting virus infection in a mammalian subject, comprising providing to said subject an effective amount of an anti-TSG101 antibody, wherein said antibody binds TSG101 present on cells of said subject which may become infected with said virus.

5

2. The method of Claim 1, wherein the anti-TSG101 antibody is a monoclonal antibody.

3. The method of Claim 2, wherein the monoclonal antibody is selected from the group consisting of antibodies C, D1, E, SG1 and CB8. .

4. The method of Claim 2, wherein the monoclonal antibody binds to an epitope in the C-terminal region of TSG101.

10

5. The method of Claim 1, wherein the anti-TSG101 antibody is a humanized antibody.

6. The method of Claim 5, wherein the humanized antibody comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOS:30-41.

7. The method of Claim 6, wherein the humanized antibody comprises amino acid sequences recited in SEQ ID NOS:30-35.

15

8. The method of Claim 6, wherein the humanized antibody comprises amino acid sequences recited in SEQ ID NOS:36-41.

9. The method of Claim 1, wherein said virus is a human immunodeficiency virus, marburg virus, ebola virus, influenza virus, respiratory syncytial virus, encephalitis virus, rous sarcoma virus, lassa fever virus, hepatitis virus, Epstein-barr virus, vaccine virus, herpes virus or

20   junin virus.

10. The method of Claim 1, wherein said mammal is a non-human and said virus is pseudorabies virus, pseudorabies virus II, swine rotavirus, swine parvovirus, bovine viral diarrhea virus, Newcastle disease virus h, swine flu virus, swine flu virus, foot and mouth disease virus, hog colera virus, swine influenza virus, African swine fever virus, infectious bovine rhinotracheitis virus, infectious laryngotracheitis virus, La Crosse virus, neonatal calf diarrhea virus, Venezuelan equine encephalomyelitis virus, punta toro virus, murine leukemia virus, mouse mammary tumor virus, equine influenza virus or equine herpesvirus, bovine respiratory syncytial virus, bovine parainfluenza virus, PRRS virus, porcine or bovine circoviruses, porcine or bovine coronaviruses, porcine or bovine RSV, porcine, bovine or avian influenza, EIAV, or bluetongue.

11. The method of claim 1, wherein said anti-TSG101 antibody is provided to said mammal by administering said antibody to dais mammal.

12. The method of claim 1, where said anti-TSG101 antibody is provided to said mammal by inoculating said mammal with a TSG101 immunogen that induces an immune response in said human including the preparation of antibodies which bind to an epitope of TSG101.

13. An anti-TSG101 monoclonal antibody, said antibody binds to a TSG101 C-terminal region consisting of an amino acid sequence recited in SEQ ID NO:3.

14. The anti-TSG101 monoclonal antibody of Claim 13, wherein in said monoclonal antibody comprises a polypeptide comprising an amino acid sequence recited in SEQ ID NO: 19, 22, 25 or 28.

15. A monoclonal antibody that binds to TSG101 selected from the group consisting of antibodies C, D1, E, 3G1 and CB8.

16. The monoclonal antibody of Claim 14, wherein said antibody is a humanized antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:30-41.

17. A pharmaceutical composition for inhibiting viral infection in a mammal, comprising:

1) an effective amount of an anti-TSG101 antibody; and

2) a pharmaceutically acceptable carrier,

wherein said anti-TSG101 antibody binds to an epitope within the TSG101 C-terminal region consisting of the amino acid sequence recited in SEQ ID NO:3.

18. A pharmaceutical composition for inhibiting viral infection in a mammal, comprising:

1) an effective amount of an anti-TSG101 antibody; and

2) a pharmaceutically acceptable carrier,

wherein said anti-TSG101 antibody is selected from the group consisting of antibodies C, D1, E, 3G1 and CB8.

ABSTRACT

The present invention provides antibodies that bind to the C-terminal region of TSG101. The invention also provides methods of using the TSG101 antibodies for the treatment of viral infections, including HIV and Ebola virus infection.

SEQ ID NO:1
GenBank Accession No. U82130.1/GI:1772663

MAVSESQLKKMVSKYKYRDLTVRETVNVITLYKDLKPVLDSYVFNDGSSRELMNLT
GTIPVPYRGNTYNIPICLWLLDTYPYNPPICFVKPTSSMTIKTGKHVDANGKIYLPYLH
EWKHPQSDLLGLIQVMIVVFGDEPPVFSRPISASYPPYQATGPPNTSYMPGMPGGI
SPYPSGYPPNPSGYPGCPYPPGGPYPATTSSQYPSQPPVTTVGPSRDGTISEDTIR
ASLISAVSDKLRWRMKEEMDRAQAELNALKRTEEDLKKGHQKLEEMVTRLDQEVA
EVDKNIELLKKKDEELSSALEKMENQSENNDIDEVIIPTAPLYKQILNLYAEENAIEDTI
FYLGEALRRGVIDLDVFLKHVRLLSRKQFQLRALMQKARKTAGLSDLY

FIG. 1

Effect of anti-TSG101 on MLV Vector Virus Production
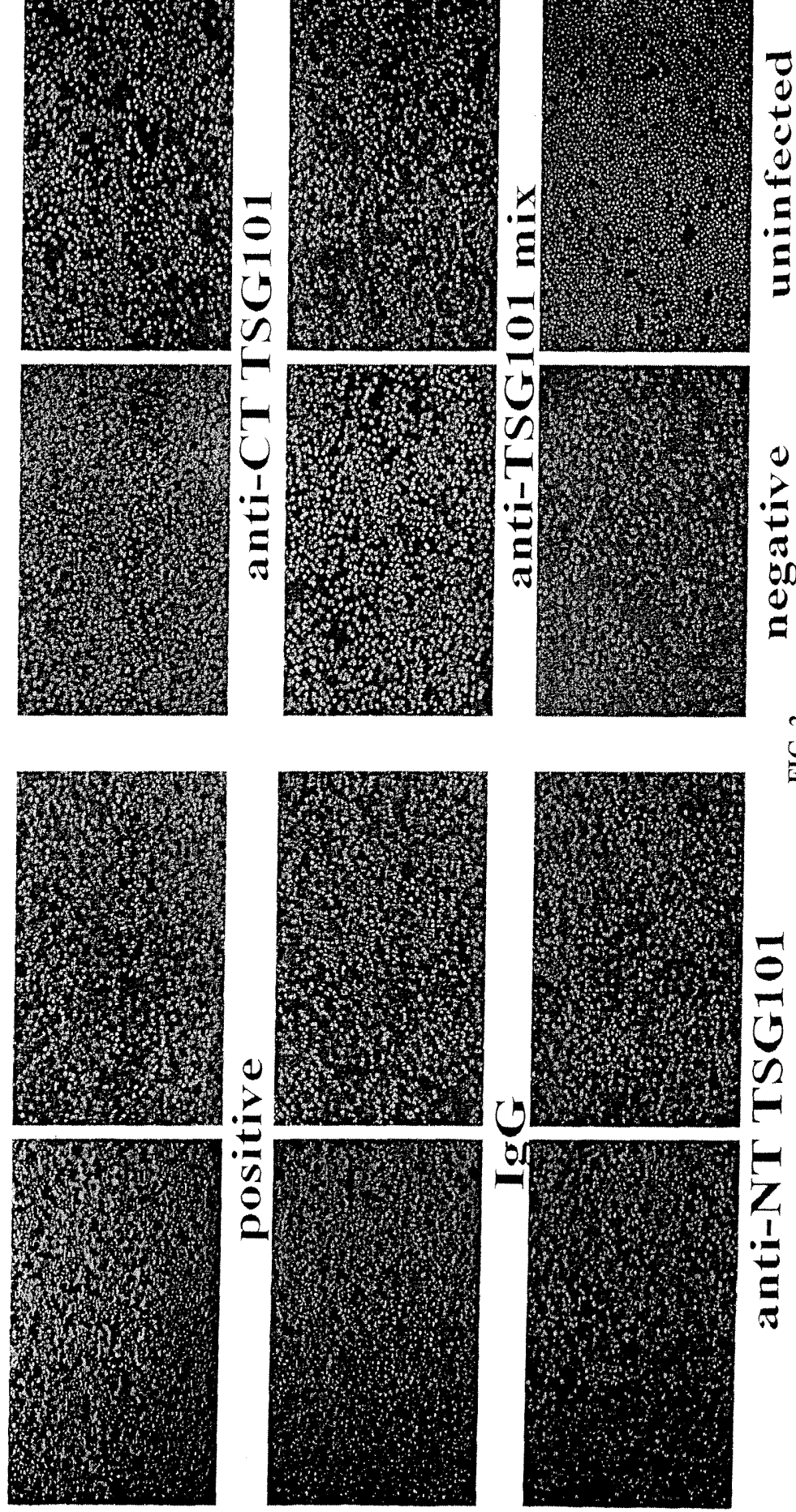
FIG. 2
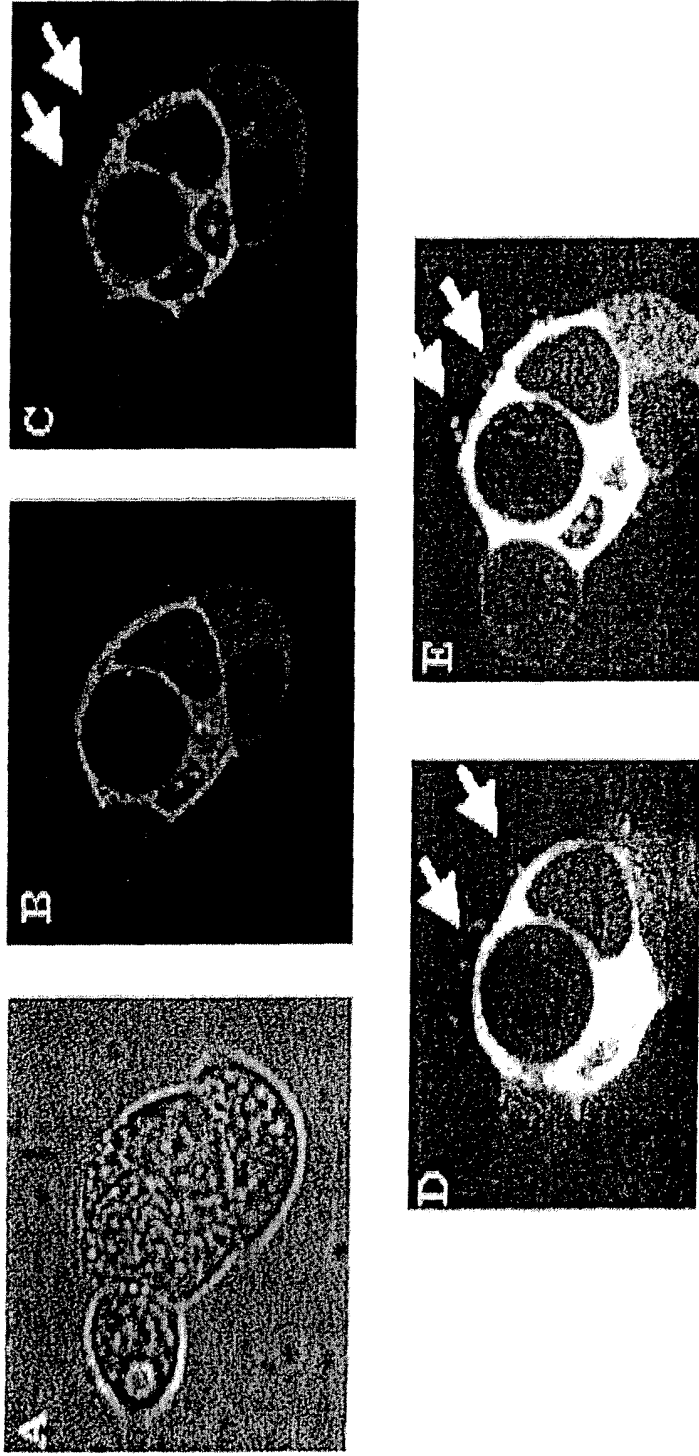
FIG. 3

H9(Control cells)
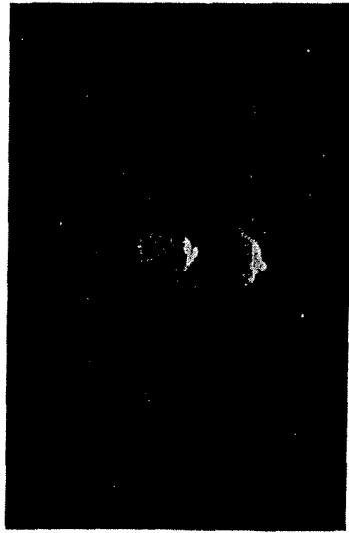
H9ΔBgl(HIV producing cells)

H9ΔBgl(HIV producing cells)
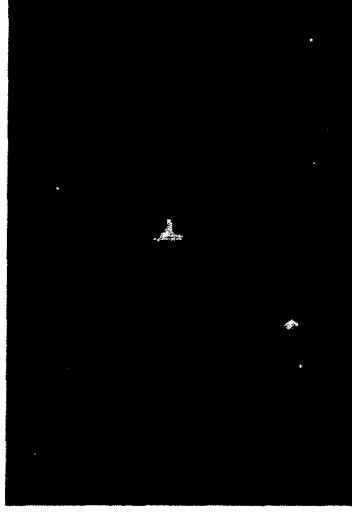
FIG. 4

FIG. 5

Cell Lysate   Virion

Pr 55 →

p24 →

1   2   3   4   5   6   7   8
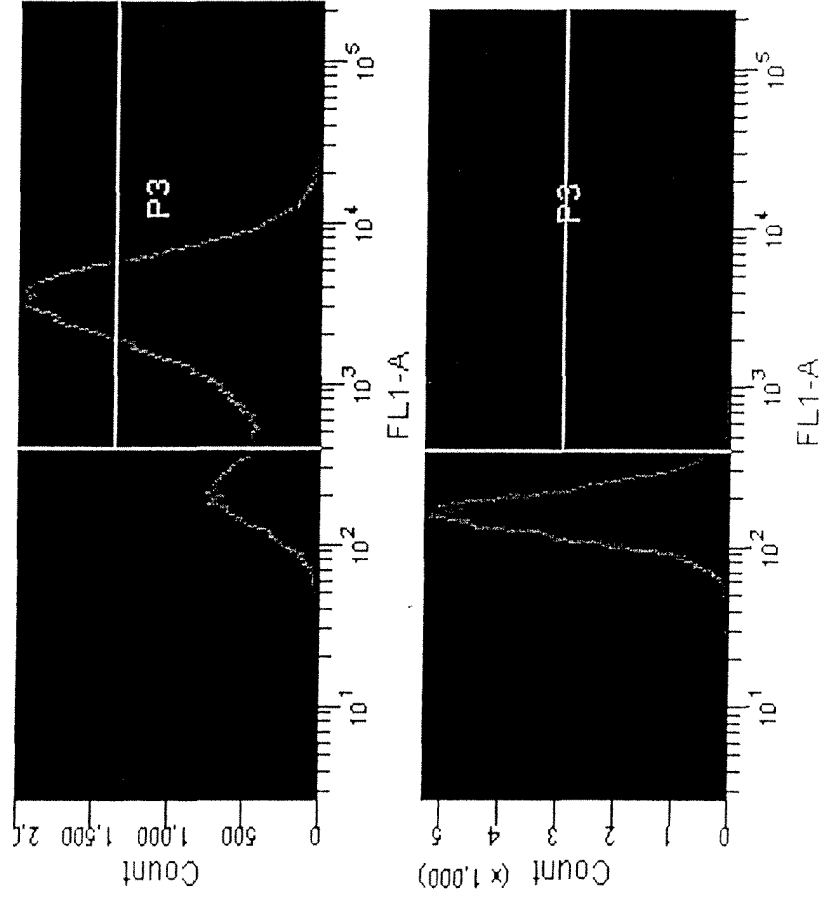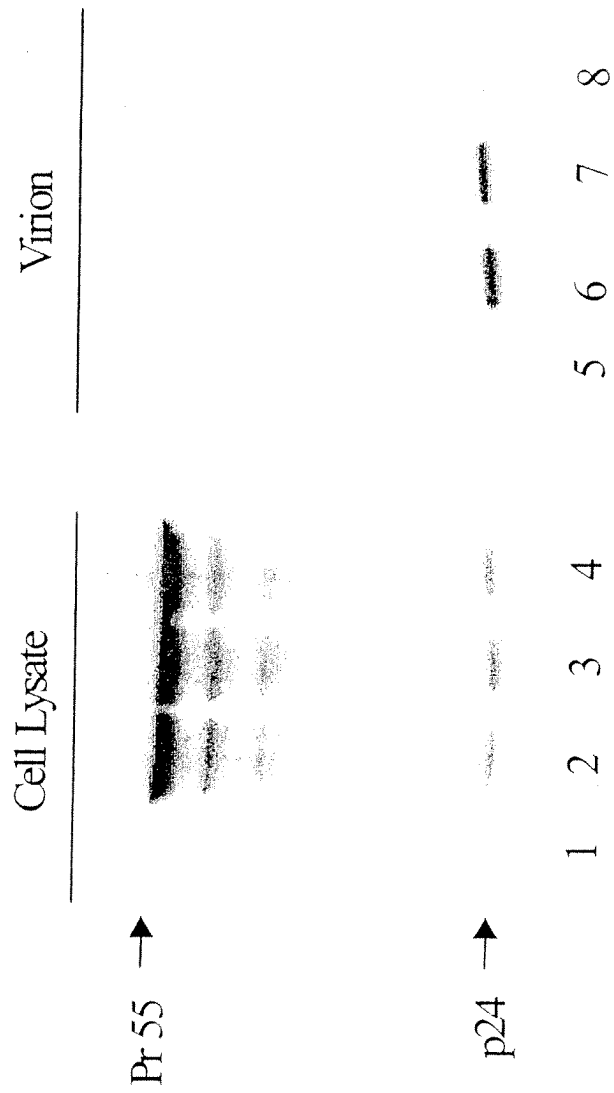
FIG. 6
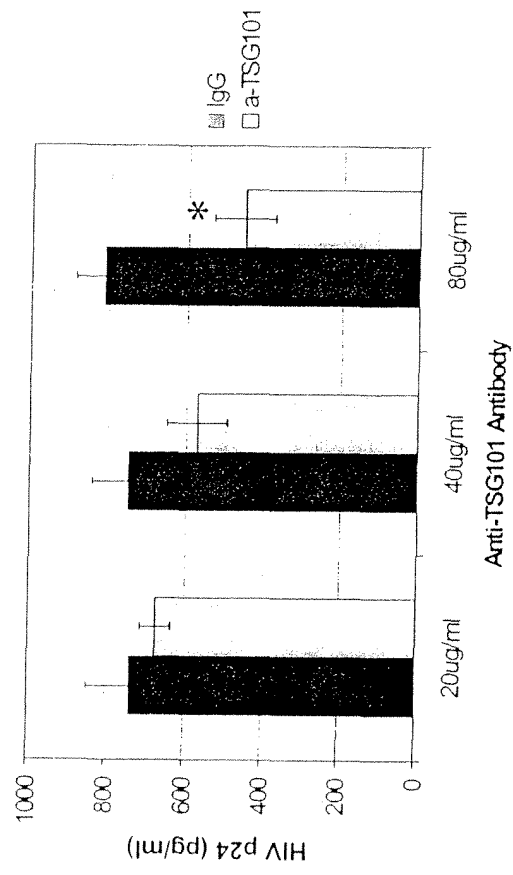
FIG. 7
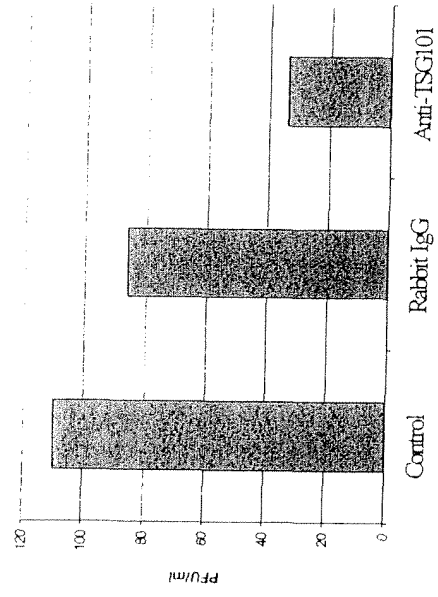
FIG. 8
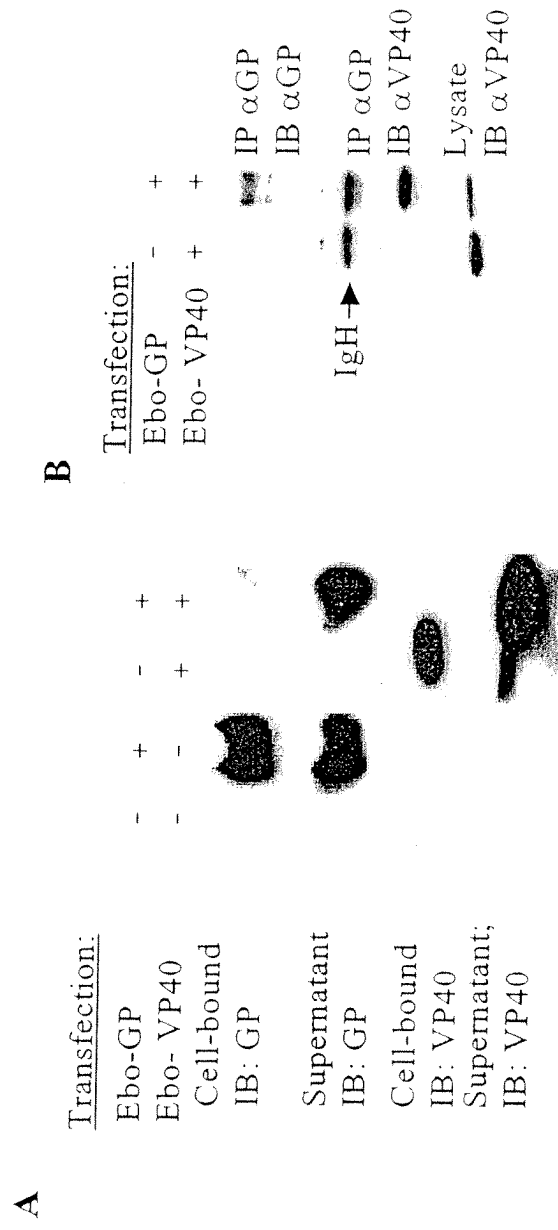
FIG. 9

A — Negative staining
B — Anti-GP immunogold staining
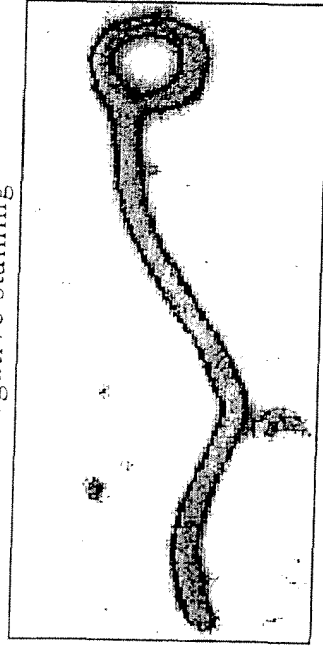
FIG. 10

FL 1- 1- 1- 300
    140 250

IP: αMyc
IB: αTSG

TSG101 FL
IgH
TSG101 (1-300)
TSG101 (1-250)

TSG101 (1-140)

IP: αMyc
IB: αVP40

IgH
VP40

Lysate
IB: αVP40

VP40

FIG. 11

Overlay with TSG101 protein

Western blot with Ebola VP40 antibody

Ebola VP40 (31-326)

Ebola VP40 (1-326)

FIG. 12

FIG. 13

FIG. 14

FIG. 15

Anti-TSG101 mAb, PE-8 Pool, Inhibits
Infectivity of Wild Type HIV (NL4-3 Strain)
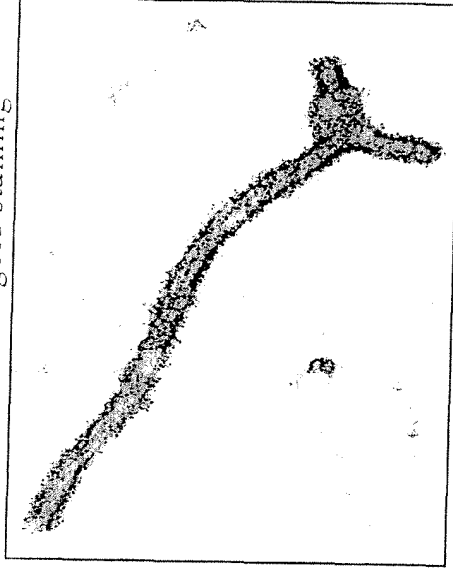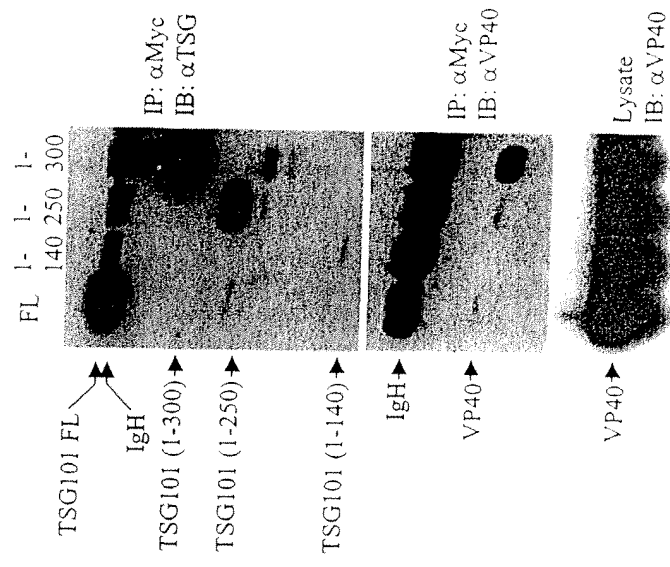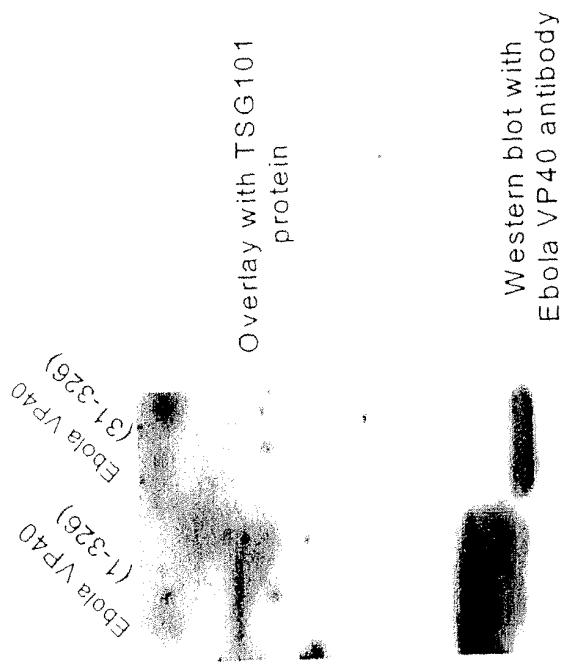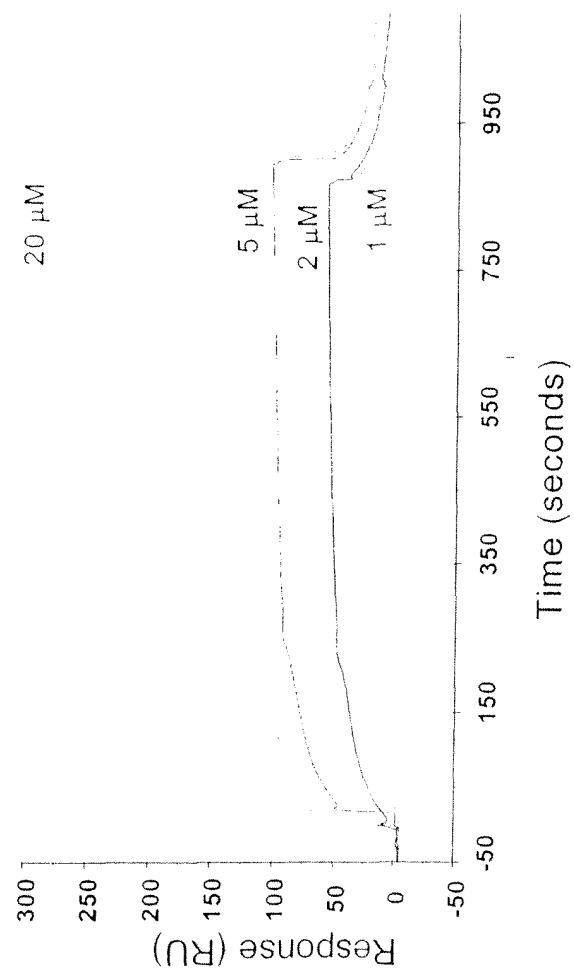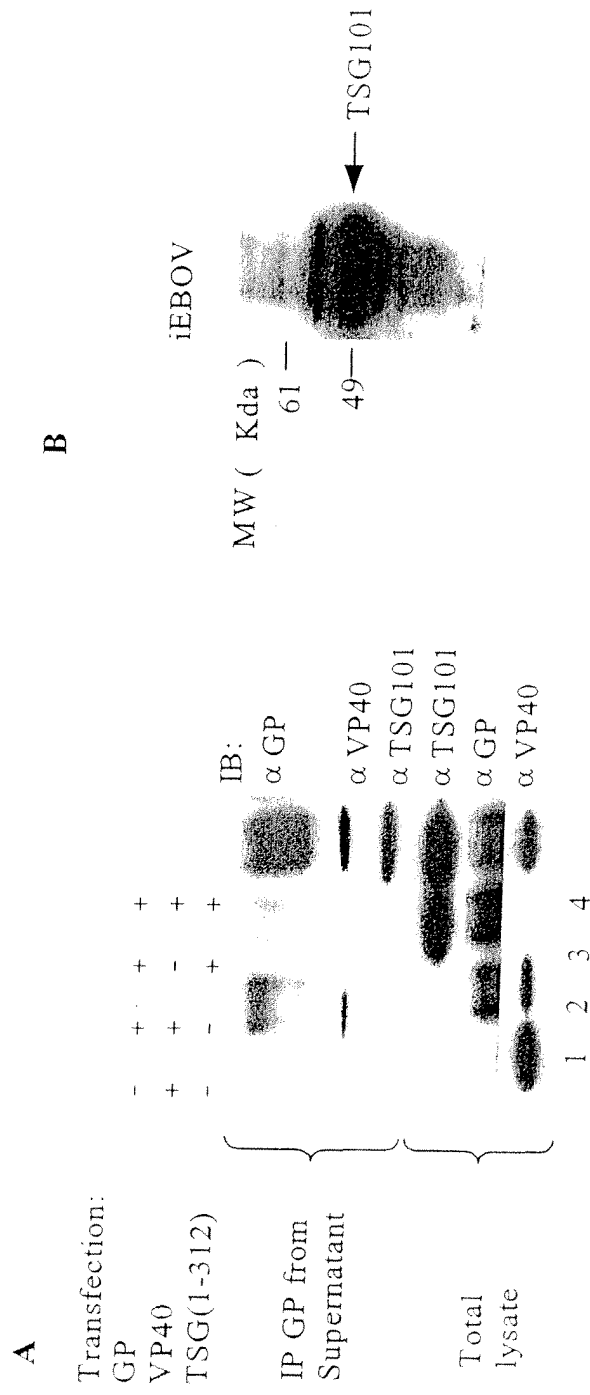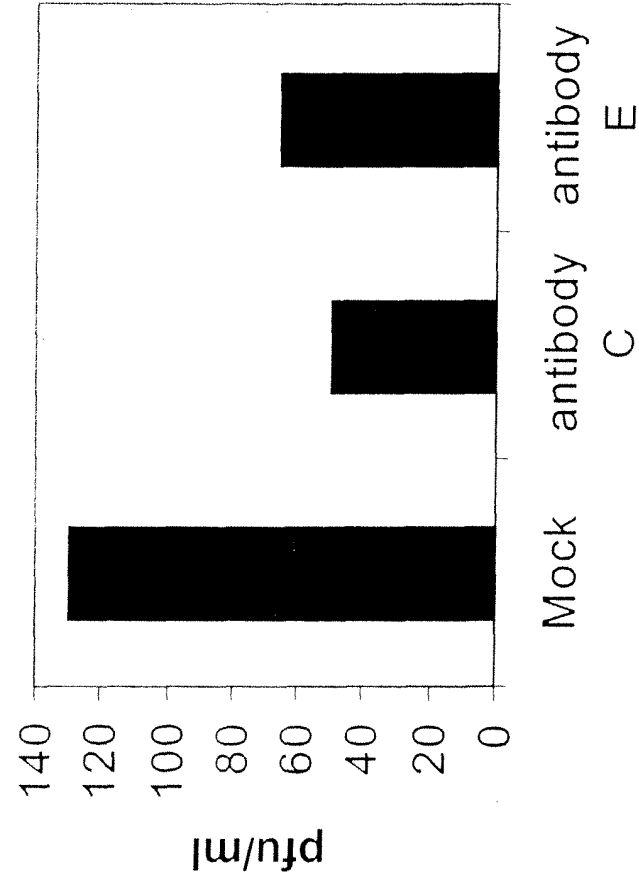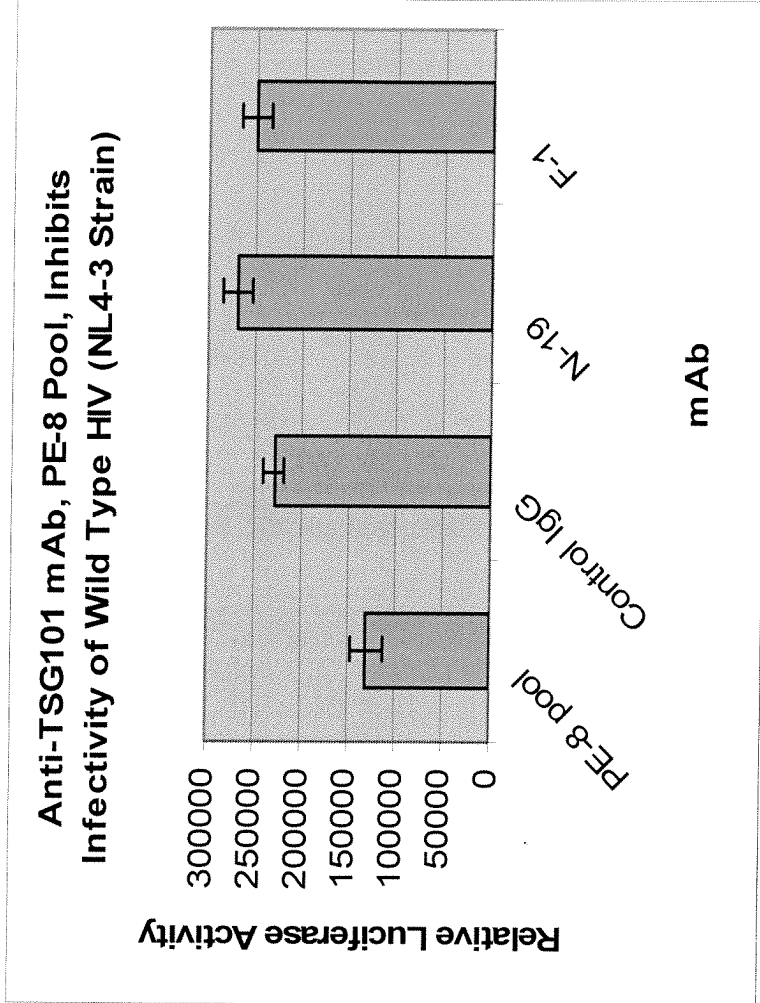
FIG. 16

Subclones of "PE-8 Pool" Anti-TSG101 mAbs Inhibit Infectivity of Wild Type HIV (NL-4-3 Strain)
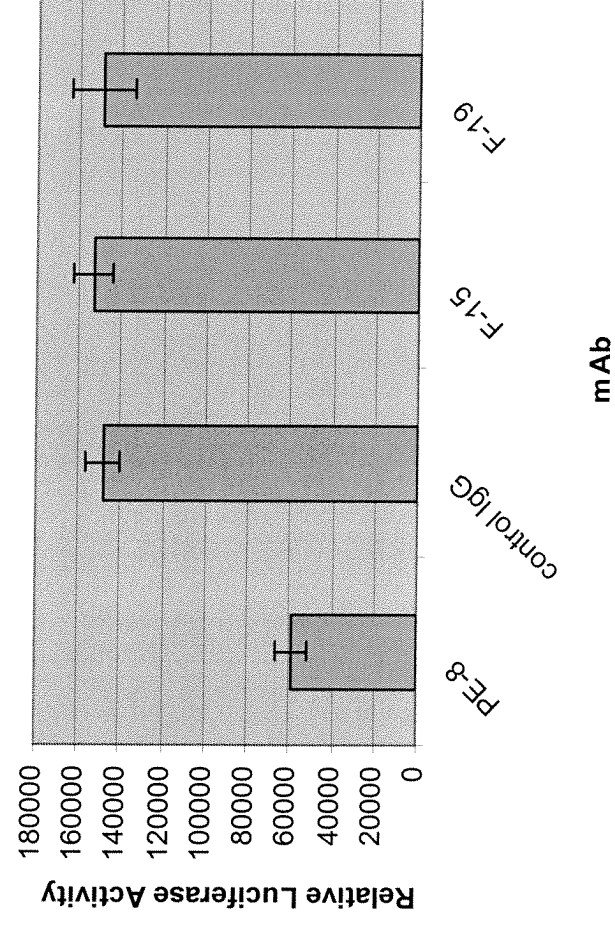
FIG. 17

A Subclone (H88) of "PE-8 Pool" Anti-TSG101 mAb Inhibits Infectivity of Wild Type HIV (NL4-3 Strain)

FIG. 18

Inhibition of Drug Resistant HIV Strains
(to Protease and RT inhibitors) by TSG101
Dominate Negative Mutant
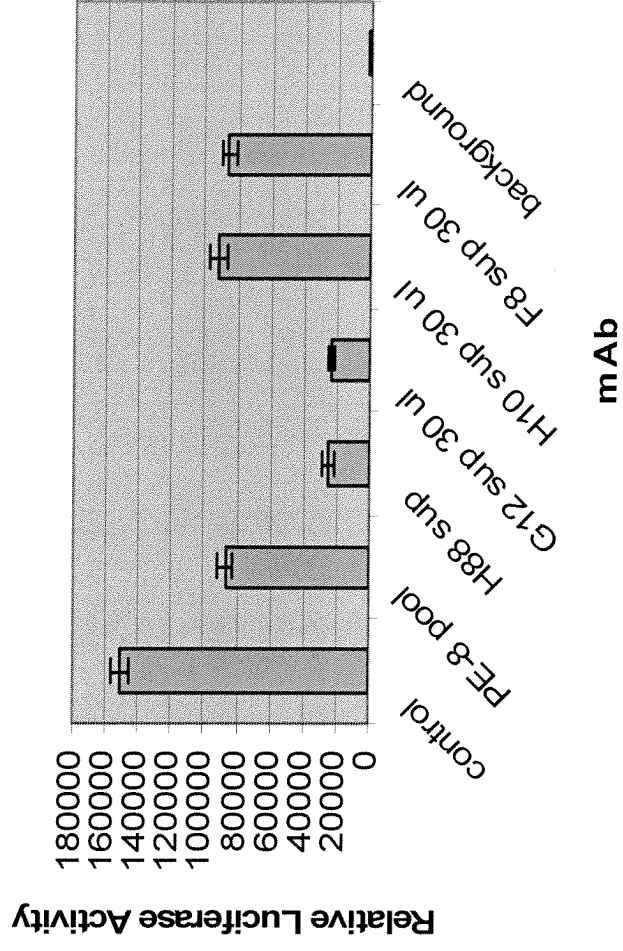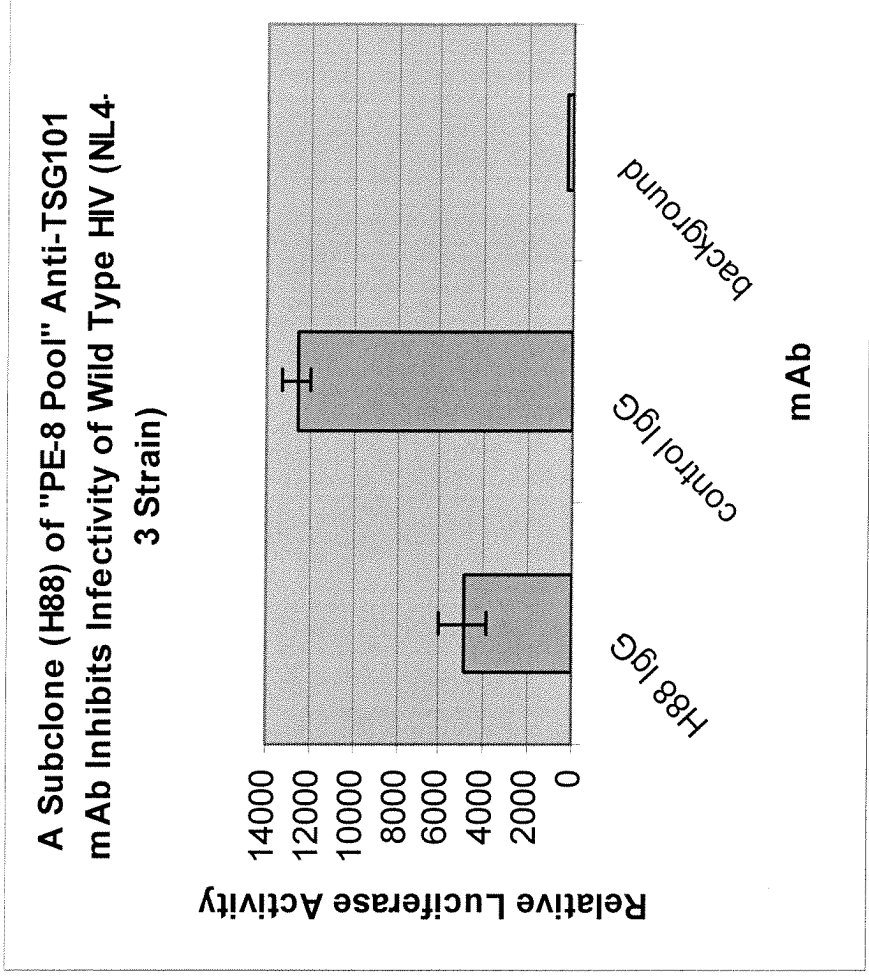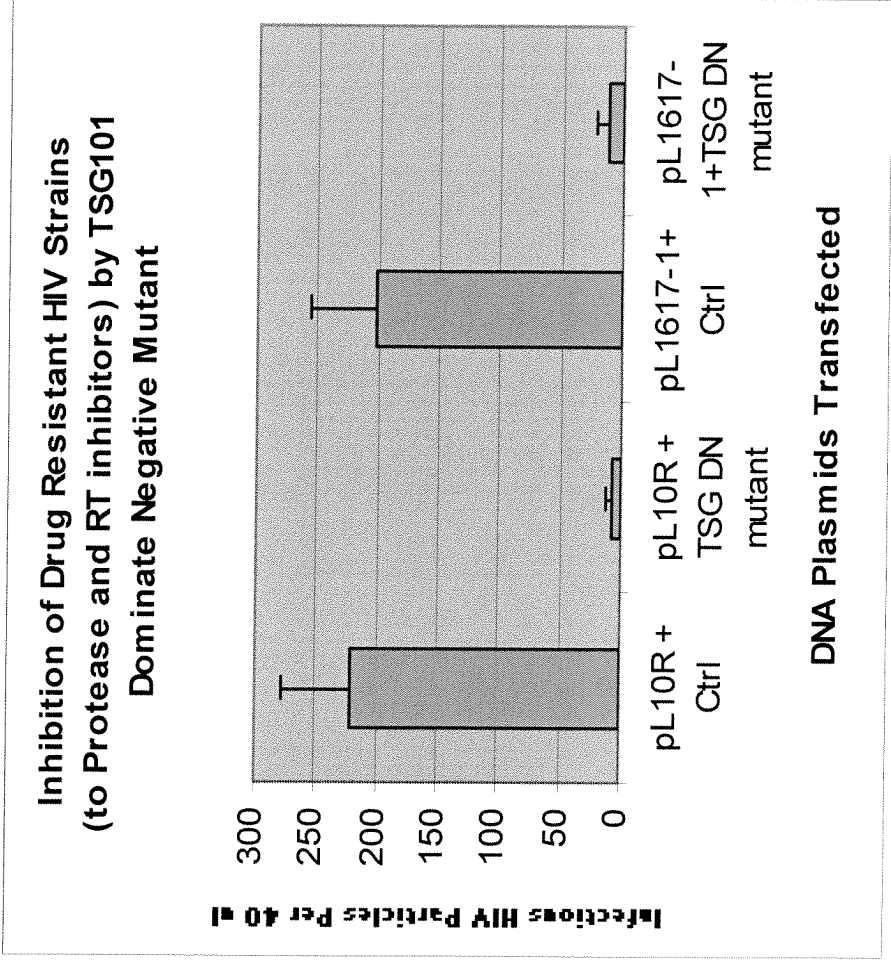
FIG. 19

Anti-TSG101 mAb Inhibits Infectivity of Drug Resistant HIV (pL10R, Protease Inhibitor Resistant Starin)

Relative Luciferase Activity
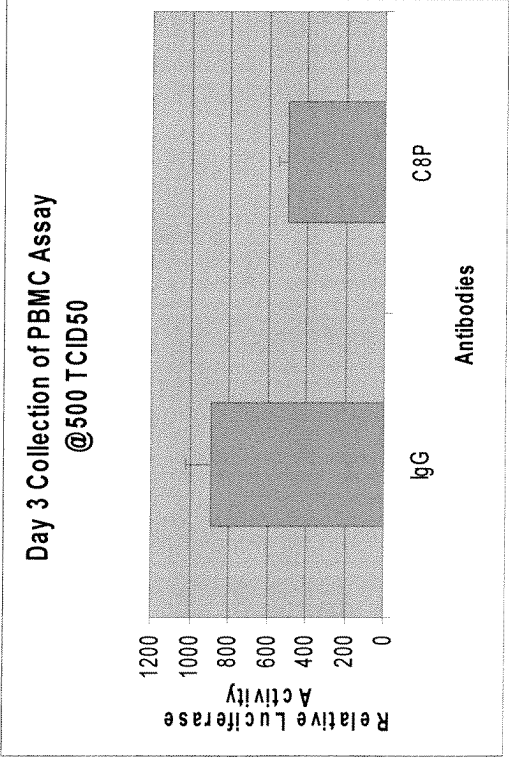
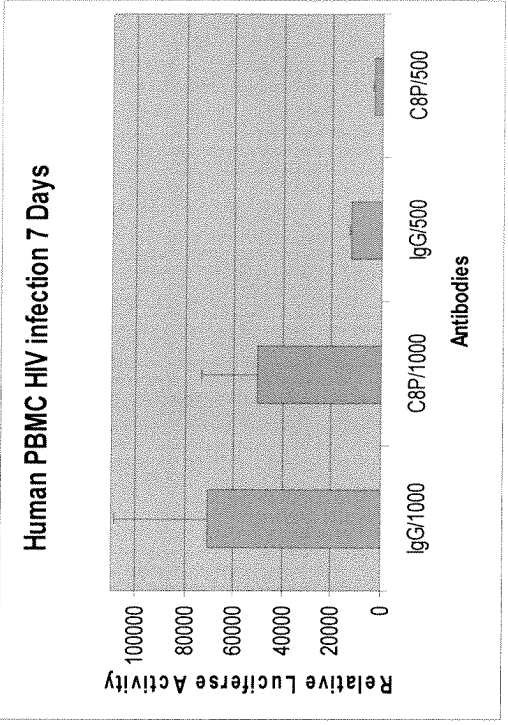mAb: PE-8, control IgG, F-15, F-19

FIG. 20

Anti-TSG101 mAb Inhibits Infectivity of
Drug Resistant HIV (p1617-1, Reverse
Transcriptase Inhibitor Resistant Strain)

FIG. 21

Human PBMC HIV infection 7 Days

FIG. 22B

Day 3 Collection of PBMC Assay
@500 TCID50
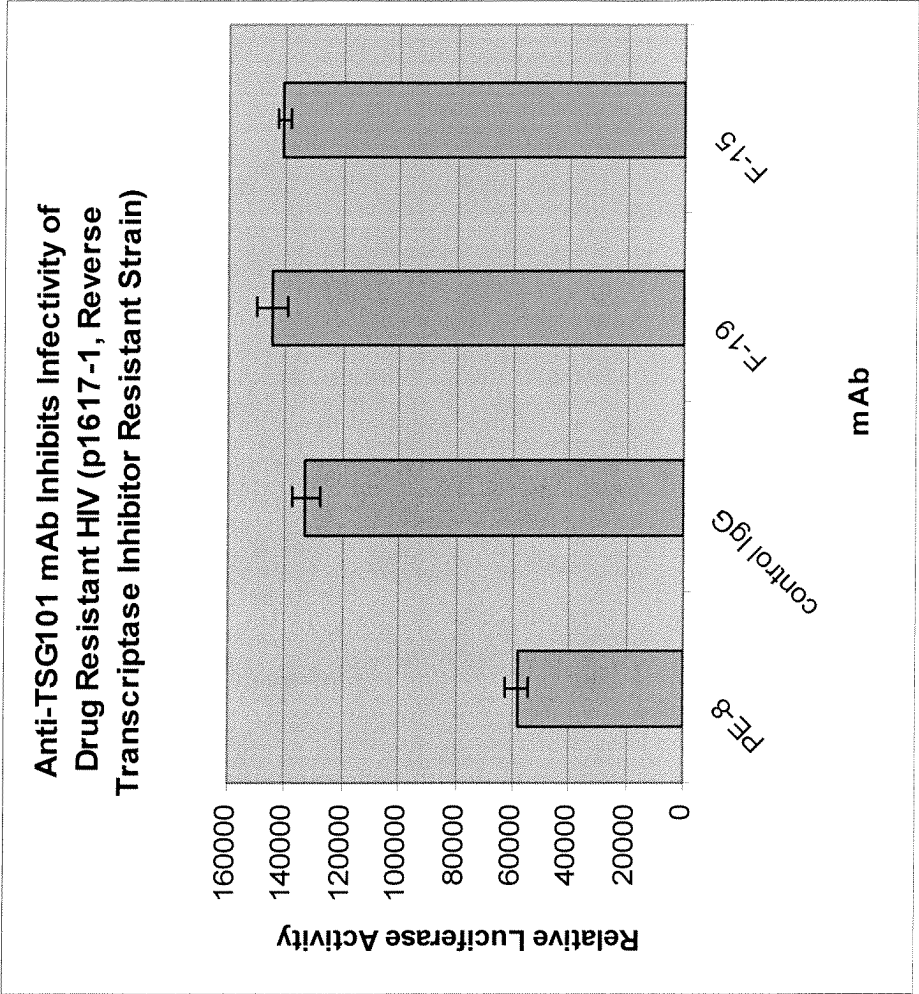
FIG. 22A

Clone D1 V$_H$ cDNA

ATGTACTTGGGACTGAGCTGTGTATTCATTGTTTTCTCTTAAAAGGTGTCCAGTGTGAG

GTGAAGCTGGATGAGACTGGAGGAGGCTTGGTGCAACCTGGGAGGCCCATGAAACTCTCG

TGTGTTGCCTCTGGATTCACTTTTAGTGACTACTGGATGAACTGGGTCCGCCAGTCTCCA

GAGAAGGGACTGGAGTGGGTAGCGCAAATTAGAAACAAACCGTTTAATTATGAAACATTT

TATTCAGATTCTGTGAAAGGCAGATTCACCATCTCAAGAGATGATTCCAAAAGTAGTGTC

TACCTGCAAATGAACAACTTAAGAAATGAGGACATGGGTATCTATTACTGTGTTCAAATCAT

AGATATGGGGTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACG

ACACCCCCATCCGTTTATCCCTTGGTCCCTGGAAGCTTGGG

>gi|110094|pir||D34903   Ig heavy chain precursor V region (9-40) - mouse
gi|639653|gb|AAA61587.1| anti-fluorescein antibody

     Length = 137

Score = 232 bits (592), Expect = 2e-60
Identities = 109/118 (92%), Positives = 114/118 (96%)

               FR1   CDR1   FR2   CDR2

Query: 1   EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPFNYET 60

Sbjct: 20 EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYET 79

               FR3   CDR3   FR4

Query: 61 FYSDSVKGRFTISRDDSKSSVYLQMNNLRNEDMGIYYCSNHRYGVAYWGQGTLVTVSA 118
           +YSDSVKGRFTISRDDSKSSVYLQMNNLR EDMGIYYC+++ Y   AYWGQGTLVTVSA
Sbjct: 80 YYSDSVKGRFTISRDDSKSSVYLQMNNLRAEDMGIYYCTSYGYHGAYWGQGTLVTVSA 137

FIG. 23

Clone D1 V$_L$ cDNA

CNGTCTGTTGCTTGCTTGTTTCAGGTACCAGAGATGTGATATCCAGATGACACAGACTACAACC

TCCCTGTCTGCCTCTCTGGGAGACAGGGTCACCATCAGTTGCAGGGCAAGTCAGGACATT

AACCATTATTTAAGCTGGTTTCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTTC

TACTCATCAAGATTACAGTCAGGTGTCCCGTCAAGGTTCAGGTGGCAGTGGGTCTGGAAGA

GATTTTCTCTCACCATTAGGGCCCTGGAACAAGAAGATATTGCCACTTACTTTTGCCAA

CAAAGTAAAGCGCTCCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCT

GATGCTGCACCAACTGTATCCATCTTCCCACCATCCAG   Length = 118

immunoglobulin light chain variable region [Mus musculus]

Score = 159 bits (401), Expect = 2e-38

Identities = 96/108 (89%), Positives = 96/108 (89%)

       FR1   CDR1   FR2   CDR2

       DIQMTQTT+SLSASLGDRVTISCRASQDI ++YL+W+QQKPDGTVKLLI +Y+SRLQSGVPS

Sbjct: 4 DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLHSGVPS 63

       FR3   CDR3   FR4

Query: 61 RFSGSGSGRDFSLTIRALEQEDIATYFCQQSKALPWTFGGGTKLEIKR 108

       RFSGSGSGRDFSLTI   LEQEDIATYFCQQ   LPWTFGGGTKLEIKR

Sbjct: 64 RFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGTKLEIKR 111

FIG. 24

Clone 3G1 V$_H$ cDNA

GCCCTTACTAGTCGACATGGGCAGGCTTACTTTCTCATTCCTGCTACTGATTGTCCCTGC

ATATGTCCTGTCCCAGGTTACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCCTCCCA

GACCCTCAGTCTGACTTGTTCTTTCTCTGGATTCTCACTGAGCACTTTAATGTGGGTGT

AGGCTGGATTCGTCAGCCATCAGGGAAGGGTCTGGAGTGGCTGGCACACATTTGGTGGGA

TAATGTCAAGCGCTATAAACCCAGCCCTGAAGAGCCGACTGACTATCTCCAAGGATACCTC

CAGCAGCCAGGTATTCCTGACATCGCCAGTGTGACACTGCAGATACTGCCACATATT

TTGTGCTCGAATAGAGAGGGGTGAATGTGTAACTACCCCTACTTTCTTACTGGGGCCAAGG

GACTCTGGTCACTGTCTCTGCAGCCAAAACGACACCCCATCCGTTATCCCCTGGCCCC

TGGAAGCTTGGG

>gi|17644233|emb|CAD18854.1| immunoglobulin heavy chain [Mus musculus]

Length = 469

Score = 250 bits (638), Expect = 1e-65
Identities = 127/156 (81%), Positives = 137/156 (87%), Gaps = 5/156 (3%)

```
                                                              FR1                      CDR1
Query: 6    MGRLTFSFLLLIVPAYVLSQVTLKESGPGILQPSQTLSLTCSFSGFSLSTFNVGVGWIRQ
Sbjct: 65   MGRLT SFLLLIVPAYVLSQVTLKESGPGILQPSQTLSLTCSFSGFSLST+ +GVGWIRQ
            MGRLTSSFLLLIVPAYVLSQVTLKESGPGILQPSQTLSLTCSFSGFSLSTYGMGVGWIRQ
Sbjct: 1
                 FR2            CDR2                                 FR3             CDR3
Query: 66   PSGKGLEWLAHIWWDNVKRYNPALKSRLTISKDTSSSQVFLDIASVDTADTATYFCARIE
Sbjct: 125  PSGKGLEWLA+IWW++ K YN ALKSRLTISKDTS++QVFL I+SVDTADTATY+CA
            PSGKGLEWLANIWWNDHKYYNSALKSRLTISKDTSNNQVFLKISSVDTADTATYYCAHY-
Sbjct: 61
                 FR4
Query: 126  GVNGNYPY-FSYWGQGTLVTVSAAKTTPPSVYPLAP 160
            NY Y   YWGQGT VTVS+AKTT PSVYPLAP
Sbjct: 120  ---SNYAYALDYWGQGTSVTVSSAKTTAPSVYPLAP 152
Query: 119
```

FIG. 25

Clone 3G1 V$_L$ cDNA

ACTAGTCGACATGGATTACAGGTGCAGATTATCAGCTTCATCGTCTAATCAGTGTCACAGT

CATATTGTCCAGTGGAGAAATTGTGGTCACCCAGTCTCCGGCACTCATGGCTGCATCTCC

AGGGGAGAGGGTCACCATCACCTGCAGTGTCAGCTCAGTGTCAGCTCAGTATAAATTCCACAACAACTTACA

CTGGTACCAACAGAAGTCAGAAGCCTCCCCCAAACCCTGGATTTATGGCACATCCAACCT

GGCTTCTGGAGTCCCTGTTCGCTTCAGTGGCAGTGGATCTGGGACCTCTTTTTCTCTCAC

AGTCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGTCAACAGTGGAGTAGTTA

CCCACTCATGACGTTCGGTGGGGGCACCAAACTGGAAATCAAGCGGGCTGATGCTGCACC

AACTGTATCCATCTTCCACCATCCAGTAAGCTTGGG

>gi|1304161|dbj|BAA03483.1| immunoglobulin gamma-3 kappa chain V-J and C
region precursor [Mus musculus]
Length = 237

Identities = 97/142 (68%), Positives = 108/142 (76%), Gaps = 1/142 (0%)

Score = 195 bits (495), Expect = 3e-49
                                              CDR1
Query: 4 MDLQVQIISFMLISVTVILSSGEIVVTQSPALMAASPGERVTITCSVSSINSNNLHWYQ 63
            MD QVQI SF+LISV+V++S GE V+TQSPA+M ASPGE+VT+TCS SS ++S N HWYQ
Sbjct: 1 MDFQVQIFSFLLISVSVLMSRGENVLTQSPAIMVASPGEKVTMTCSASSRVSSGNFHWYQ 60
                  FR2   CDR2   FR3   CDR3
Query: 64 QKSEASPKPWIYGTSNLASGVPVRFSGSGSGTSFSLTVSSMEAEDAATYYCQQWSSYPLM 123
            QK SPK WIY TS+LASGVP RFSGSGSGTS LT SSMEAEDAATYYCQQWS YP
Sbjct: 61 QKPGTSPKLWIYRTSSLASGVPARFSGSGSGTSYFLTISSMEAEDAATYYCQQWSGYP-R 119
                  FR4
Query: 124 TFGGGTKLEIKRADAAPTVSIF 145
            TFGGGTKLEIKRADAAPTVSIF
Sbjct: 120 TFGGGTKLEIKRADAAPTVSIF 141

FIG. 26

Percentage of Survival

Days Post Challenge

Mab Ctrl
C8
PBS Ctrl
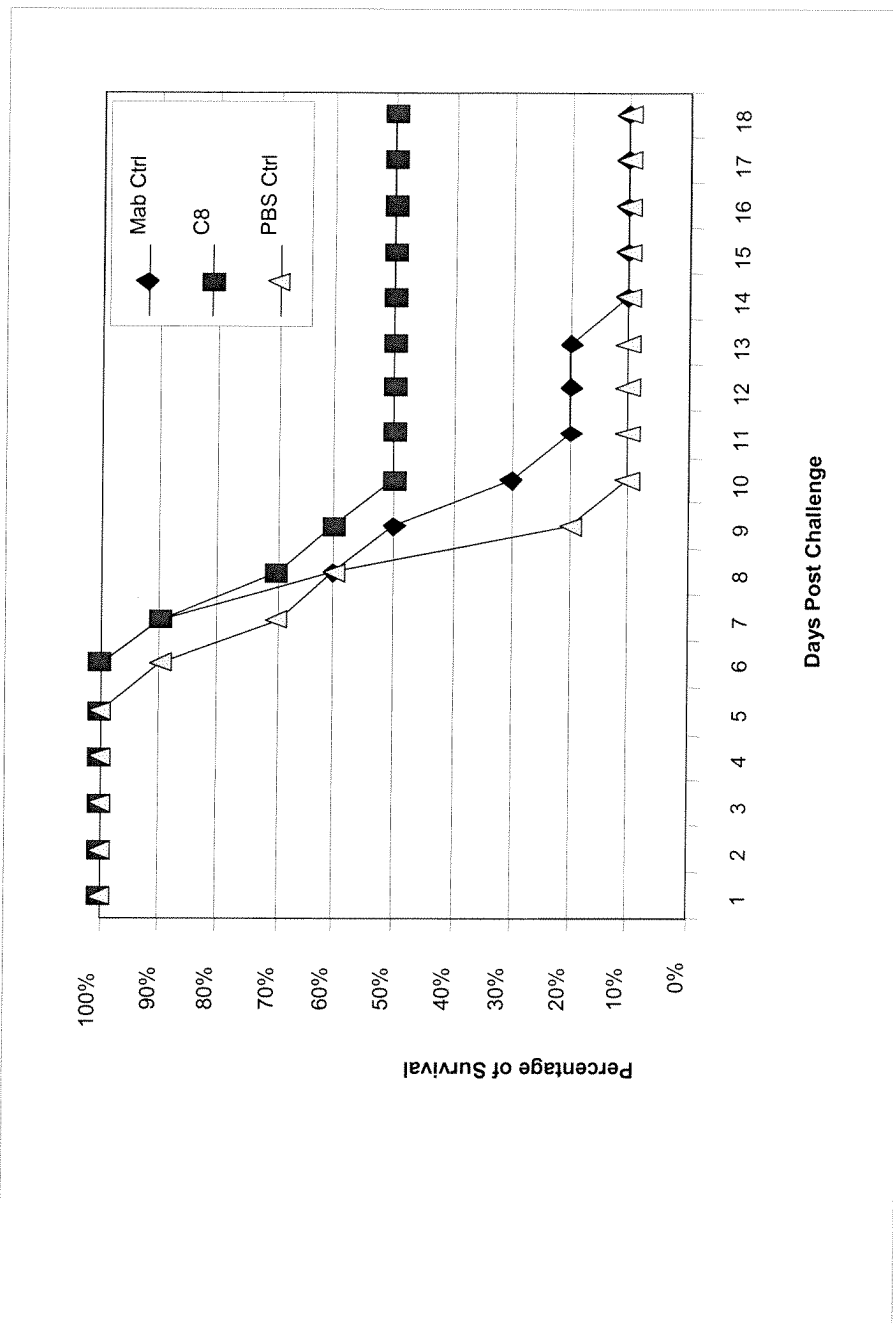
FIG. 27

Fig. 28

TSG101 Is Relevant to Many Virus Types - Broad-Spectrum Potential

HIV-1	Retrovirus	VerPlank et al (2001) *PNAS*
Ebola	Filovirus	Martin-Serrano et al (2001) *Nature Med.*
HIV-2	Retrovirus	Myers and Allen (2002) *J Virol.*
HTLV-1	Retrovirus	Bouamr et al (2003) *J Virol.*
MPMV	Retrovirus	Gottwein et al, (2003) *J Virol.*
Arenavirus	Arenavirus	Perez et al (2003) *PNAS.*
MLV	Retrovirus	Camus et al (2007) Mol Biol Cell
EIAV	Retrovirus	Shehu-Xilaga et al. (2003) *J Virol.*
Influenza – Seasonal	Orthomyxovirus	Functional Genetics, unpublished
Influenza – Pandemic	Orthomyxovirus	Functional Genetics, unpublished
Japanese Enceph.	Flavivirus	Chiou et al. (2003) *J Gen Virol.*
Foamy	Retrovirus	Patton et al. (2005) *J Virol.*
Rous Sarcoma	Retrovirus	Medina et al. (2005) *Traffic*
Lassa	Arenavirus	Urata et al. (2006) *J Virol.*
Hepatitis E	Herpevirus	Surjit et al. (2006) *J Biol Chem.*
Epstein-Barr	Herpevirus	Chua et al., (2007) *J Virol.*
Marburg	Filovirus	Urata et al. (2007) *J. Virol.*
Respiratory Syncytial	Paramyxovirus	Functional Genetics, unpublished
Vaccinia	Orthopoxvirus	Honeychurch et al. (2007) *J. Virol.*
Bluetongue	Orbivirus	Wirblich et al. (2006) *J Virol.*
Human Herpesvirus 6A	Herpesviridae	Hammarstedt et al. (2007) *J Virol.*
Junin	Arenavirus	Ellenberg et al. (2007) J. Gen Virol.

Legend:
Group I: ds DNA
Group III: ds RNA
Group IV: (+) ssRNA
Group V: (-) ssRNA
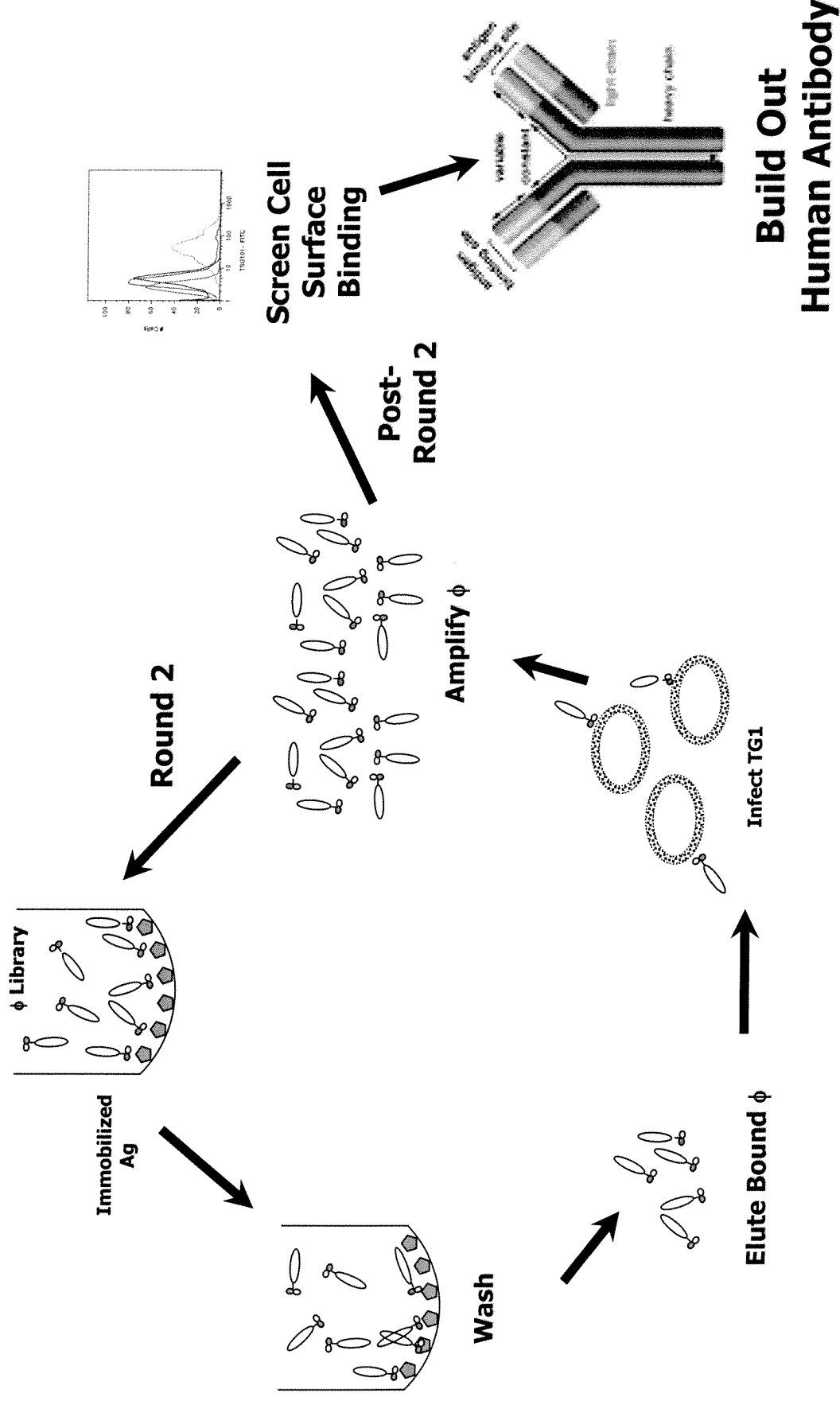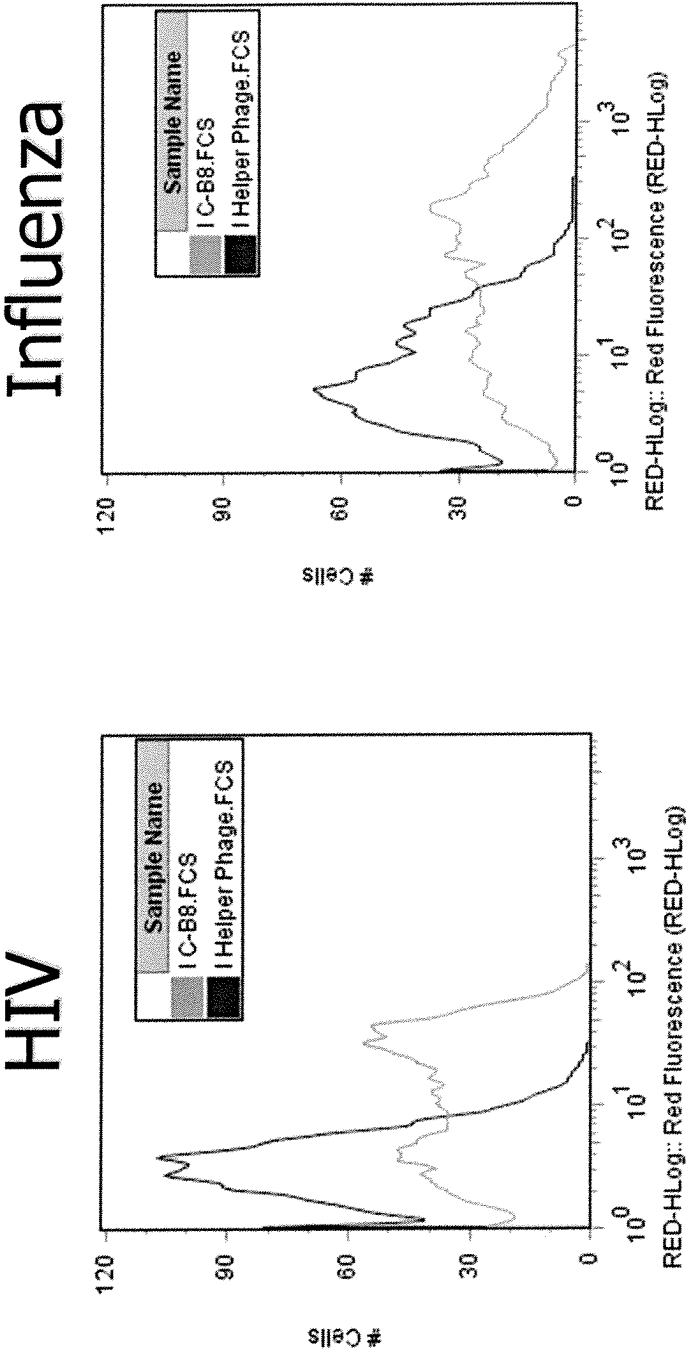Group VI: ssRNA-RT

Fig. 29

Screening Human scFv
Phage library

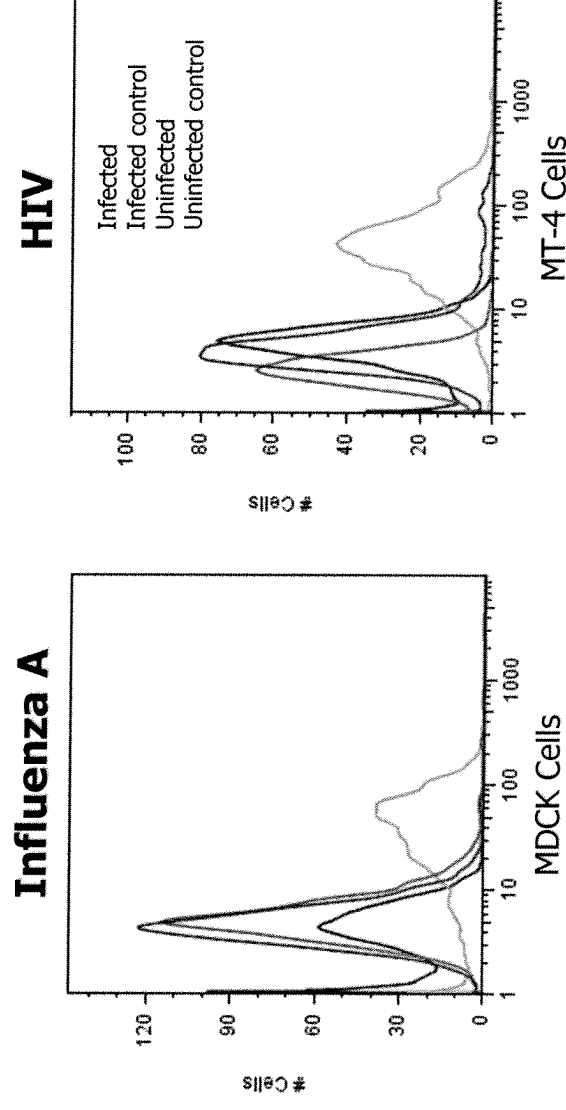## Fig. 30

FGI-101
Phage Candidates (Representative)

HIV   Influenza

Note: RSV Testing
In Progress

Experimental Design:
- **Phage Identity:** CB8
- **TSG101 mAb:** CB8
- **Host:** A/Udorn-Infected MDCK or HIV-1-infected MT-4 Cells

Fig. 31

TSG101 Antibody Candidates Screening Strategy

Influenza A

HIV

Infected
Infected control
Uninfected
Uninfected control

Cells

MDCK Cells

Cells

MT-4 Cells

Conclusion: The Same TSG101 Antibodies Can Recognize Cells Infected with Different Viruses
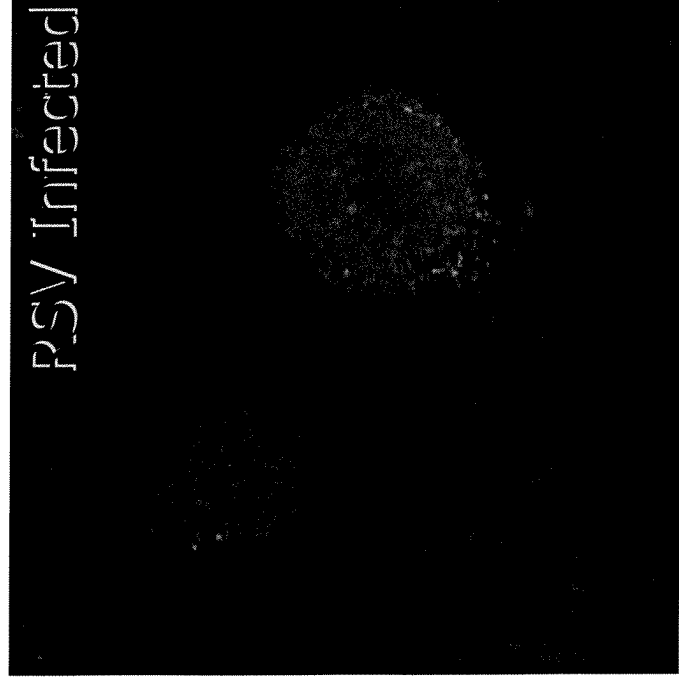
Experimental Design:
- **TSG101 mAb:** CB8
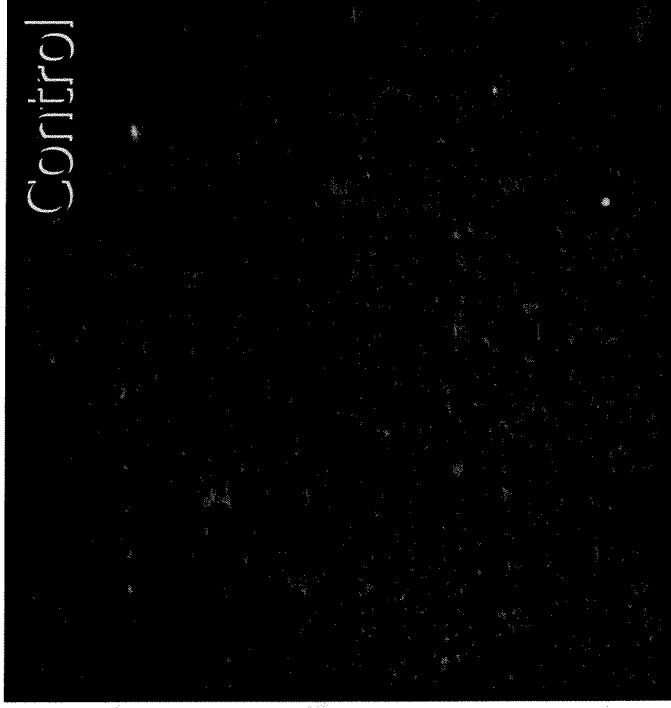- **Host:** A/Udorn-Infected MDCK or HIV-1-infected MT-4 Cells

Fig. 32

Broad-Spectrum Potential
Respiratory Viruses

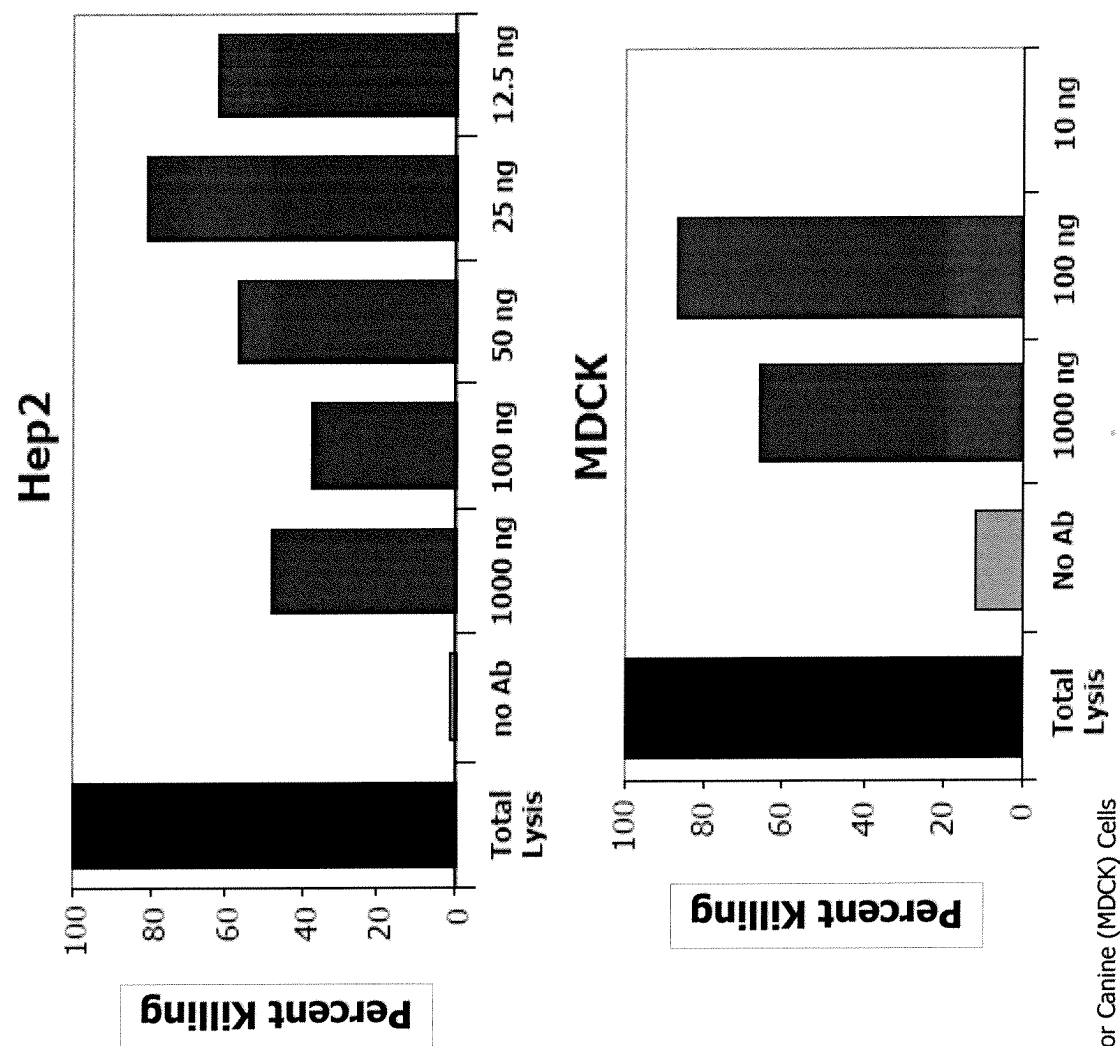Experiment: TSG101 mAb Staining of RSV-Infected Cells

RSV Infected   Control

Nuclear DNA
RFP-RSV
Surface TSG101

Conclusion: TSG101 Presents An Opportunity for Simultaneous
Prophylaxis/Therapy for Influenza and RSV

Fig. 33

TSG101 mAbs Kill Infected Cells

Mechanism #1: Antibody Dependent Cellular Cytotoxicity (ADCC)

Experimental Design:
- **TSG101 mAb:** CB8
- **Host:** A/Udorn-Infected Human (Hep2) or Canine (MDCK) Cells
- **Conditions:** t=4h, E:T = 3:1

Fig. 34

TSG101 mAbs Inhibit Infection
Mechanism #2: Direct Biological Activity